

AD_____

Award Number: W81XWH-10-1-0598

TITLE: Role of IKK-alpha in EGFR Signaling Regulation

PRINCIPAL INVESTIGATOR: Chia-Wei Li, Ph.D.

CONTRACTING ORGANIZATION: M.D. Anderson Cancer Center, University of Texas
Houston, TX 77030

REPORT DATE: September 2013

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE September 2013		2. REPORT TYPE Annual Summary		3. DATES COVERED 15 August 2012 – 14 August 2013	
4. TITLE AND SUBTITLE Role of IKK-alpha in EGFR Signaling Regulation				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-10-1-0598	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Chia-Wei Li, Ph.D. E-Mail: cwli@mdanderson.org				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) M.D. Anderson Cancer Center, University of Texas Houston, TX 77030				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Overexpression of EGFR is frequently linked to more aggressive tumor behavior, including increased proliferation, metastasis, and therapeutic resistance. Here, we identified a molecular linkage between IKK α and EGFR signaling in breast cancer cells. Inhibition of IKKs activity elevates EGFR tyrosine phosphorylation. In addition, IKK α forms a specific interaction with EGFR in Golgi apparatus and catalyzes EGFR S1026 phosphorylation. We found that EGFR S1026A possess a stronger tumorigenesis phenotype compare with wild type EGFR suggesting a negative regulation of IKK α in EGFR signaling. In agreement with an earlier finding where conditional ablation of IKK α in the mice keratinocytes elevates the autocrine loop of EGFR, our results further provide a potent role of IKK α kinase activity in preservation of EGFR activity.					
15. SUBJECT TERMS EGFR signaling, inflammatory kinase, IKKalpha, tumorigenesis, tumor suppressor					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			USAMRMC
			UU	21	19b. TELEPHONE NUMBER (include area code)

Table of Contents

Page

Introduction.....	1
Body.....	1-5
Conclusions.....	5-6
Future Works.....	6
Key Research Accomplishments.....	6-7
Reportable Outcomes.....	7
References.....	7-8
Appendices.....	8

Title: The role of IKK α in EGFR signaling regulation

1. INTRODUCTION:

A Triple-negative breast cancer (TNBC), which account for approximately 15-20% of breast cancers in the United States, lacks the expression of estrogen receptor (ER) and progesterone receptor (PR) as well as amplification of HER2/neu and is associated with poorer outcome compared with other breast cancer subtypes (1-3). TNBC also overlaps with the basal-like breast cancer, which is a subtype of breast cancer classified by genomic signatures identified in the molecular classification, although they are not same (2, 4). Unlike ER-positive, PR-positive, or HER2-overexpressing tumors, the lack of well-defined molecular targets and the heterogeneity of the disease pose a challenge for treating TNBC (1, 3).

Aberrant activation and overexpression of the epidermal growth factor receptor (EGFR) contribute to aggressive tumor behavior and poor patient prognosis (5), and thus drugs that target EGFR are being used to treat many types of cancers. However, they are not as effective for breast cancer, suggesting that other mechanisms (6, 7) or biological functions of EGFR that have yet to be discovered may have important roles in breast cancer. Overexpression of EGFR has been frequently observed in TNBC and is associated with poor clinical outcome in TNBC patients (4, 8). These findings suggest that further understanding of the role of EGFR is critical for implementing successful anti-EGFR therapy in TNBC.

In this study, we found the inflammation regulator, IKK α , inhibits EGFR activity through a novel signaling pathway in breast cancer cells. IKK α binds to and phosphorylated EGFR at S1026. Inhibition of IKK activity led to hyperphosphorylation of EGFR Y845 and STAT3 705 suggesting a negative regulatory role of IKK α in breast cancer cells. Interestingly, low IKK α expression and high STAT3 activation was found in TNBC cells. These result suggest deregulation of IKK α contribute to the aggressive phenotype of TNBC cell. Altogether, our study provides novel mechanistic insight of IKK α mediated EGFR suppression in TNBC cells.

2. RESEARCH ACCOMPLISHMENTS BODY

Part I: Clinical relevance of pEGFR S1026 and p-STAT3

To recapitulate IKK α mediated EGFR S1026 phosphorylation, we purified and analyzed the phospho-EGFR S1026 antibody. As shown in the second year's progress report, IKK α induces a nice phosphorylation of EGFR using a p-EGFR S1026 antibody. Mutation of S1026 to alanine (S1026A) abolishes IKK α mediated EGFR phosphorylation. The purified anti-phospho-S1026 EGFR antibody specifically recognized phospho-EGFR^{WT} but not EGFR^{S1026A} (Fig. 1A). To further determine the clinical relevance, we analyzed the correlation between phospho-S1026 EGFR and phosphor-Y705 STAT3 in human breast cancer tissues. A negative correlation was identified between phospho-S1026 EGFR and phosphor-Y705 STAT3 in human TNBC but not non-TNBC tissues (Fig. 1B and 1C). In addition, low level of phospho-S1026 and high level of phosphor-Y705 STAT3 correlated with a poor survival in TNBC patients cohorts (data not shown) suggesting their important roles in TNBC proliferation.

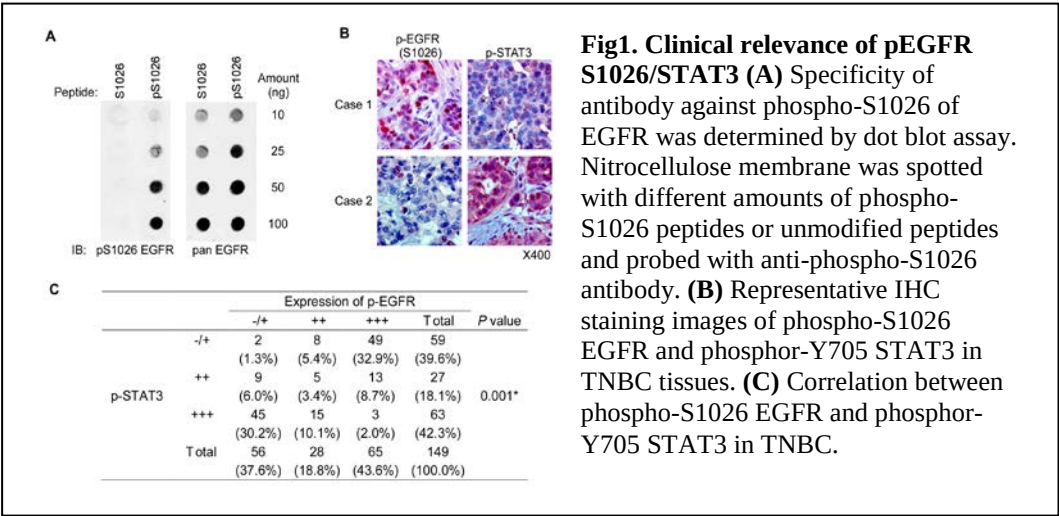


Fig1. Clinical relevance of pEGFR S1026/STAT3 (A) Specificity of antibody against phospho-S1026 of EGFR was determined by dot blot assay. Nitrocellulose membrane was spotted with different amounts of phospho-S1026 peptides or unmodified peptides and probed with anti-phospho-S1026 antibody. (B) Representative IHC staining images of phospho-S1026 EGFR and phosphor-Y705 STAT3 in TNBC tissues. (C) Correlation between phospho-S1026 EGFR and phosphor-Y705 STAT3 in TNBC.

Part II: IKKα is downregulated in Triple Negative Breast Cancer (TNBC)

As we report in the last year's progress report, we examine gene expression profile of IKKα (NCBI gene ID: Chuk) using public data set (CCLE)(9) to identify the potential STAT3 downstream target that regulated by IKKα. We compared the expression profile of IKKα and 60 STAT3 downstream targets in breast cancer cells (10). Among then, 12 genes show negatively correlated with IKKα expression using CCLE. Nonsupervised hierarchical clustering analysis was performed based on Erbb2, ERα (ESR1), PR (Pgr) profile. Strikingly, the gene list was able to distinguish basal-like from luminal type breast cancer cells with high accuracy (90% properly segregated) (Fig. 2A). To our surprise, IKKα expression was found to be downregulated in TNBC cells (Fig. 2B). We also analyzed an earlier identified STAT3 target regulated by IKKα through EGFR, CCL2. Consistently, CCL2 was found to be upregulated in TNBC cells (Fig. 2C).

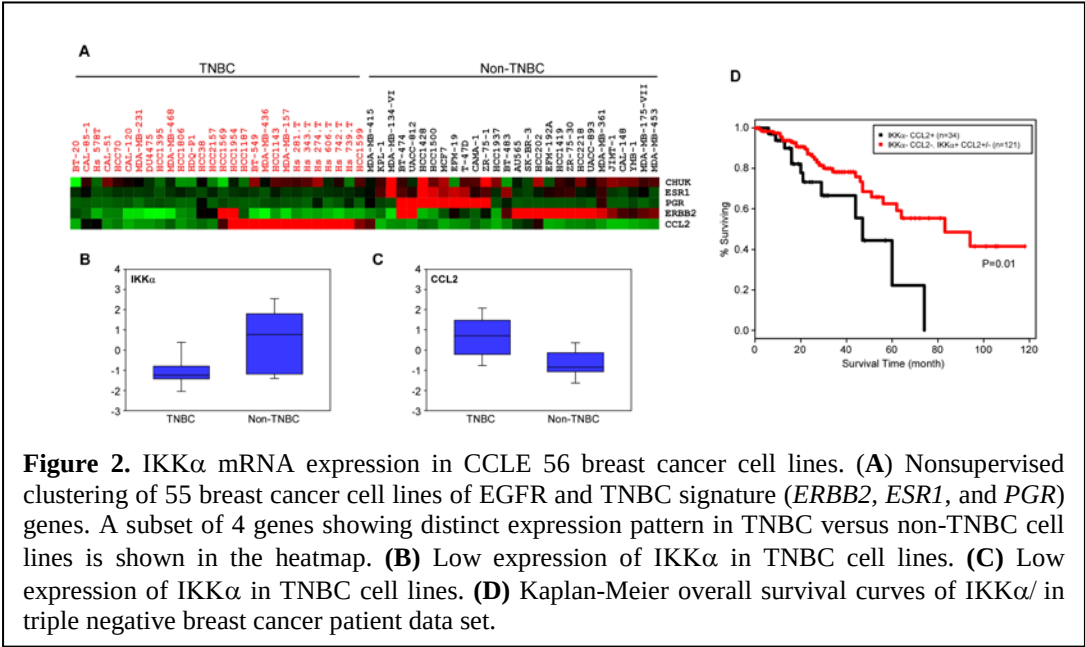
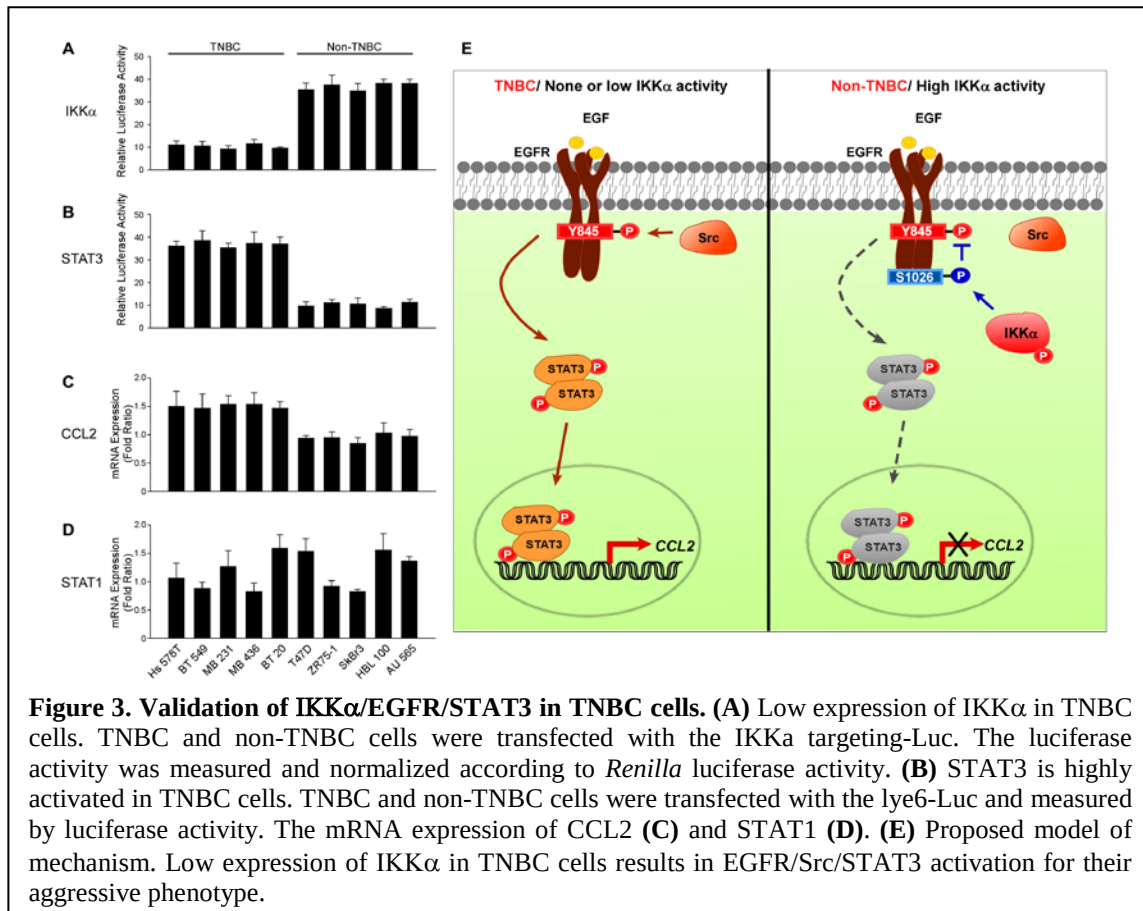


Figure 2. IKKα mRNA expression in CCLE 56 breast cancer cell lines. (A) Nonsupervised clustering of 55 breast cancer cell lines of EGFR and TNBC signature (*ERBB2*, *ESR1*, and *PGR*) genes. A subset of 4 genes showing distinct expression pattern in TNBC versus non-TNBC cell lines is shown in the heatmap. (B) Low expression of IKKα in TNBC cell lines. (C) Low expression of IKKα in TNBC cell lines. (D) Kaplan-Meier overall survival curves of IKKα/ in triple negative breast cancer patient data set.

We next asked if clinical distinct group of patient samples also shared the differential expression pattern of IKK α . First, we analyzed IKK α and CCL2 genes expression from Netherlands Cancer Institute (NKI) data set, $n=295$ (11). To do this, patients in the NKI cohort were first dichotomized according to their expression levels. As expected, low IKK α and high CCL2 of TNBC patients showed a significant difference in recurrence-free survival (RFS) compare to the rest of the status. Altogether, downregulation of IKK α and high expression of CCL2 is likely contributed to TNBC aggressive phenotype.

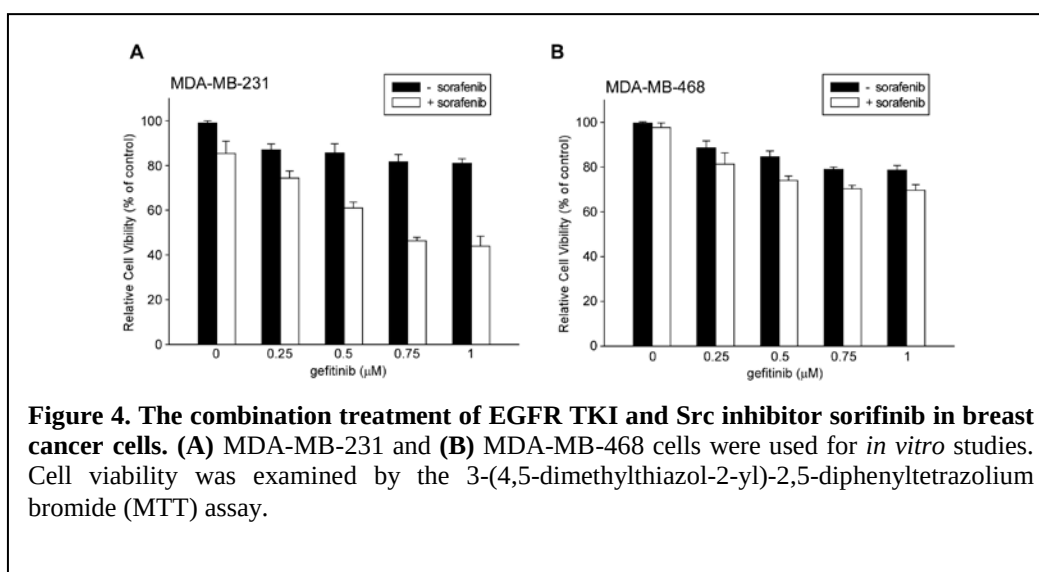
Part III: Downregulation of IKK α in Triple Negative Breast Cancer (TNBC)

Based on the data set mining, the newly identified mechanism is likely to be TNBC specific. To further validate the results from the database analysis, we analyzed the intrinsic IKK α /STAT3 activity and the alteration of mRNA expression in TNBC and non-TNBC cells. Consistently, TNBC cells show relatively low IKK α and high STAT3 activity using a reporter assay (Fig. 3A and 3B). Interestingly, the levels of mRNA expression CCL2 (Fig. 3C) but not STAT1 (Fig. 3D) is significantly increased in those TNBC cells. Together, these data indicate that IKK α mediated EGFR phosphorylation and inhibition is restricted in TNBC cells. Downregulation of IKK α in TNBC cells results in the activation of EGFR/Src/STAT3 loop and therefore activates STAT3 downstream target CCL2 (Fig. 3E).



Part IV: inhibition of Src activity sensitize TNBC cells to TKI

Sorafenib (Nexavar, BAY43-9006), a multi-kinase inhibitor, inhibits Src mediated STAT3 activation in many type of cancer cells. However, sorafenib alone is not effective in breast cancer cells and has an unacceptable toxicity at high doses. Because gefitinib alone is not enough to kill breast cancer cells due to reported resistance in breast cancer cells, we tested the combination of sorafenib and gefitinib in to determine if sorafenib sensitizes TNBC cells to gefitinib. The combined treatment of gefitinib and sorafenib synergistically suppressed cellular proliferation in TNBC cells such as MDA-MB-231 and MDA-MB-468 (Fig. 4A and 4B) but not in non-TNBC cells such as MCF7 and T47D cells compared to either inhibitor alone (data not shown).



Part V: Tumor necrosis factor alpha-induces EMT required p65-mediated transcriptional upregulation of Twist1. Supported by DoD funding, the PI has accomplished another project unraveling tumor microenvironment-mediated breast cancer metastasis.

In the past three DoD funding years, we also identify that chronic exposure of pro-inflammatory cytokine, $\text{TNF}\alpha$, in induces breast cancer cells EMT phenotypic changes and stemness, and subsequently identified Twist1 as a novel modulator of this regulation (12). Our results unravel the $\text{NF}\kappa\text{B}$ -mediated Twist1 upregulation as a novel therapeutic strategy for breast cancer treatment. In fact, this report has brought a lot of attention in the cancer research field. One year non-self citation of this particular paper is now exceeding 30 times.

Part VI: Phosphorylation of Twist1 by AKT1 Modulates Epithelial-Mesenchyme Transition in Breast Cancer Cells. Supported by DoD funding, the PI also serves as first author of another manuscript related to Triple Negative Breast Cancer (TNBC)

treatment (see attached abstract and figures).

Accumulating evidence from both cellular and genetic studies suggests AKT1/PKB α serves as a negative regulator of EMT during breast cancer metastasis. In this study, we found that AKT1 induced a phosphorylation-dependent ubiquitination and degradation of Twist1, engages the proteasome to Twist1-mediated EMT regulation. Our findings reveal a novel molecular concept by which non-specific inhibition of AKT may result in Twist1 stabilization to increase the metastatic potential in breast cancer cells. This manuscript is now under revised in Cancer Cell. The PI has used the past five month to address reviewer's questions and is now ready to response to Cancer Cell.

Part VII: EGFR Associates with and Primes GSK3 β for its Inactivation and Mcl-1 upregulation. Supported by DoD funding, the PI also serves as first author of another manuscript related to Triple Negative Breast Cancer (TNBC) treatment (see attached abstract).

In studies with endogenous GSK3 β association complex, we identified EGFR as a novel GSK3 β -interacting protein, which phosphorylates GSK3 β and inhibits GSK3 β activation. We revealed that GSK3 β 's activity is stringently modulated by a previously unknown and reversible modification, ubiquitination through a distinct TRAF6 binding motif of GSK3 β . The essence of PE motif for enhancing GSK3 β activity suggested TRAF6-mediated K63 ubiquitination is involved. Furthermore, TRAF6 activates GSK3 β activity, thereby affecting GSK3 β dependent apoptosis. Altogether, we demonstrate EGFR associates with and phosphorylates GSK3 β , which primed inactivation of GSK3 β by inhibiting TRAF6-mediated ubiquitination, resulting in Mcl-1 upregulation.

3. CONCLUSION

EGFR, as an essential growth and survival factor, plays an important role in many cancer types. The modification patterns of EGFR are critical for its function and the understanding of these EGFR modifications could help us design the optimal therapeutic strategies for targeting various EGFR-associated cancers and/or non-cancerous diseases. We herein identified a novel posttranslational modification of EGFR which plays an indispensable role in regulation of EGFR signaling pathways. We found that IKK α is responsible for EGFR S1026 serine phosphorylation. S1026 phosphorylation of EGFR negatively impacts its synergic interaction with Src. Similar to other serine/threonine phosphorylation on the EGFR, phosphorylation by IKK α downregulates EGFR signaling and thereby diminishes cell growth and tumorigenesis.

The third year research is focusing on the pathological identification of the novel signaling in breast cancer cells. Using CCLE dataset analysis, we first identified a negative correlation of IKK α and TNBC cells. We went on to identify CCL2 as a novel downstream target of IKK α /EGFR/STAT3 signaling axis. Biochemical analysis consolidates the involvement of CCL2 in TNBC cells. We also analysis two patient data set and found out the poor prognosis of low IKK α and high CCL2. This result provides the first evidence showing the inhibitory nature of IKK α in both cell based study and patient samples.

In conclusion, the proposed experiments by the PI have accomplished. In the past three years, the PI identified the novel phosphorylation on EGFR by IKK α at S1026. Functional analysis indicate IKK α trigger a negative regulation by interfering EGFR/Src interaction. Database analysis further suggests the inhibitory nature of IKK α in TNBC cells and therefore possesses high level of STAT3 activity and CCL2 expression. In addition, we also breed MMTV-IKK $\alpha^{-/-}$ /EGFR mice in FVB background. Indeed, our preliminary data indicate that mice lack of IKK α accelerate hyperplastic lesion (please second year report). As the animal facility at MD Anderson cancer center has recently undergo rederivation process, we have request no cost extension to collect more MMTV-IKK $\alpha^{-/-}$ /EGFR mice to reach statistical significance.

4. FUTURE WORKS:

The MMTV-hEGFR transgenic mice developed mammary epithelial hyperplasias, hypertrophy, or slight dysplasias in about 55% of mammary glands of animals examined. Since the inhibition of IKK α results in hyperactivation of EGFR to provide a survival advantage for cancer cells, we plan to create conditional knock out of IKK α in mammary gland and cross with EGFR overexpression mice to measure tumor onset. The age of the mouse in which mammary tumor is first palpable will be recorded and tumor size will be measured. Although our preliminary data indicate that mice lack of IKK α accelerate hyperplastic lesion, deletion of IKK α enhances EGFR mediated tumorigenesis remains unknown. We are now breeding more IKK $\alpha^{-/-}$ /EGFR mice to reach statistical significance.

5. NO COST EXTENSION:

To ensure adequate completion of the originally approved project, the PI requested no cost extension of grant number **W81XWH-10-1-0598** for a period of twelve months, commencing (09/14/2013) and ending on (09/14/2014).

As the animal facility of MDACC underwent conversion to specific-pathogen-free (SPF) status similar to the CSPF status we had in the basement of the BSRB. The institution required that all animals in this area that need to be maintained undergo a rederivation process to clean up the strain. This work has done by our Genetically Engineered Mouse Facility (GEMF) and the whole rederivation process has taken over twelve month to finish. Owing to this unexpected situation, the PI needs to collect the rest of the data of transgenic mice experiments. Once the results are collected, the data will be evaluated for publication. Therefore, an extended research period is required.

6. KEY RESEARCH ACCOMPLISHMENTS: 2012-2013

- a) Complete biological function of EGFR S1026A *in vivo*. New data is not included in the third year report.
- b) Identification of IKK α as negative regulator in Triple Negative Breast Cancer cells.

- c) Characterization of phospho-EGFR S1026 antibody. EGFR S1026 phosphorylation is negative correlated with p-STAT3 in TNBC cells
- d) Identify CCL2 as IKK α /EGFR/STAT3 downstream target. CCL2 is highly expressed in the TNBC cells.
- e) Sorafenib and Gefitinib show combinatory effect in treating TNBC cells.
- f) Two papers related to Triple Negative Breast Cancer (PI is the first author) have either been revised or ready for submission.

7. REPORTABLE OUTCOMES 2012-2013

Li CW, Xia W, Huo L, Lim SO, Wu Y, Hsu JL, Chao CH, Yamaguchi H, Yang NK, Ding Q, Wang Y, Lai YJ, Labaff AM, Wu TJ, Lin BR, Yang MH, Hortobagyi GN, Hung MC. (2012) *Epithelial-Mesenchymal Transition Induced by TNF- α Requires NF- κ B-Mediated Transcriptional Upregulation of Twist1*. **Cancer Research** 72(5): 1290-1300

Wang Y, Ding Q, Yen CJ, Xia W, Izzo JG, Lang JY, **Li CW**, Hsu JL, Miller SA, Wang X, Lee DF, Hsu JM, Huo L, Labaff AM, Liu D, Huang TH, Lai CC, Tsai FJ, Chang WC, Chen CH, Wu TT, Buttar NS, Wang KK, Wu Y, Wang H, Ajani J, Hung MC. (2012) *The crosstalk of mTOR/S6K1 and Hedgehog pathways*. **Cancer Cell**. 20; 21(3):374-87.

Hsu JL, **Li CW**, Hsu YH, Wang Y, Hung MC. (2013) “*Cancer gene therapy by cancer-specific and cancer-targeting promoters*” in *Cancer Gene Therapy by Viral and Non-viral Vectors* (edited by Brenner MK). **Springer**, In press.

Li CW, Lai CC, He X, Chao CH, Sun HL, Yamaguchi H, Xia W, Wang Y, Chou CK, Hsu JM, Lee DF, Ding Q, Wang H, Labaff A, Hung MC. (2013) *Phosphorylation of Twist1 at multiple sites by AKT1 modulating EMT in breast cancer cells*. **Revised in Cancer Cell**.

Li CW, Ding Q, Xia W, Lai CC, Wang Y, Huo L, Li HJ, Lamothe B, Campos AD, Hur L, Lai YJ, Darnay BG and Hung MC. (2013) *The role of IKK α in EGFR signaling regulation*. *In submission*

8. REFERENCE:

1. B. D. Lehmann *et al.*, Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *The Journal of clinical investigation* **121**, 2750 (Jul, 2011).
2. E. A. Rakha, J. S. Reis-Filho, I. O. Ellis, Basal-like breast cancer: a critical review. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **26**, 2568 (May 20, 2008).
3. O. Metzger-Filho *et al.*, Dissecting the heterogeneity of triple-negative breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical*

- Oncology* **30**, 1879 (May 20, 2012).
4. P. Gazinska *et al.*, Comparison of basal-like triple-negative breast cancer defined by morphology, immunohistochemistry and transcriptional profiles. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc.* (Feb 8, 2013).
 5. G. Lurje, H. J. Lenz, EGFR signaling and drug discovery. *Oncology* **77**, 400 (2009).
 6. M. N. Dickler, M. A. Cobleigh, K. D. Miller, P. M. Klein, E. P. Winer, Efficacy and safety of erlotinib in patients with locally advanced or metastatic breast cancer. *Breast cancer research and treatment* **115**, 115 (May, 2009).
 7. K. L. Mueller, Z. Q. Yang, R. Haddad, S. P. Ethier, J. L. Boerner, EGFR/Met association regulates EGFR TKI resistance in breast cancer. *J Mol Signal* **5**, 8 (2010).
 8. L. Carey, E. Winer, G. Viale, D. Cameron, L. Gianni, Triple-negative breast cancer: disease entity or title of convenience? *Nature reviews. Clinical oncology* **7**, 683 (Dec, 2010).
 9. Barretina, J., Caponigro, G., Stransky, N., Venkatesan, K., Margolin, A. A., Kim, S., Wilson, C. J., Lehar, J., Kryukov, G. V., Sonkin, D., Reddy, A., Liu, M., Murray, L., Berger, M. F., Monahan, J. E., Morais, P., Meltzer, J., Korejwa, A., Jane-Valbuena, J., Mapa, F. A., Thibault, J., Bric-Furlong, E., Raman, P., Shipway, A., Engels, I. H., Cheng, J., Yu, G. K., Yu, J., Aspesi, P., Jr., de Silva, M., Jagtap, K., Jones, M. D., Wang, L., Hatton, C., Palescandolo, E., Gupta, S., Mahan, S., Sougnez, C., Onofrio, R. C., Liefeld, T., MacConaill, L., Winckler, W., Reich, M., Li, N., Mesirov, J. P., Gabriel, S. B., Getz, G., Ardlie, K., Chan, V., Myer, V. E., Weber, B. L., Porter, J., Warmuth, M., Finan, P., Harris, J. L., Meyerson, M., Golub, T. R., Morrissey, M. P., Sellers, W. R., Schlegel, R., and Garraway, L. A. (2012) *Nature* **483**, 603-607
 10. Dauer, D. J., Ferraro, B., Song, L., Yu, B., Mora, L., Buettner, R., Enkemann, S., Jove, R., and Haura, E. B. (2005) *Oncogene* **24**, 3397-3408
 11. van de Vijver, M. J., He, Y. D., van't Veer, L. J., Dai, H., Hart, A. A., Voskuil, D. W., Schreiber, G. J., Peterse, J. L., Roberts, C., Marton, M. J., Parrish, M., Atsma, D., Witteveen, A., Glas, A., Delahaye, L., van der Velde, T., Bartelink, H., Rodenhuis, S., Rutgers, E. T., Friend, S. H., and Bernards, R. (2002) *The New England journal of medicine* **347**, 1999-2009
 12. Li CW, Xia W, Huo L, Lim SO, Wu Y, Hsu JL, Chao CH, Yamaguchi H, Yang NK, Ding Q, Wang Y, Lai YJ, Labaff AM, Wu TJ, Lin BR, Yang MH, Hortobagyi GN, Hung MC, Epithelial-Mesenchymal Transition Induced by TNF- α Requires NF- κ B-Mediated Transcriptional Upregulation of Twist1. *Cancer Research* **72**, 1290 (2012).

9. APPENDICES:

A. Abstract and Figures of recent manuscript revised in Cancer Cell

AKT1-mediated Inhibition of Breast Cancer Epithelial-Mesenchyme Transition Requires Phosphorylation-dependent Twist1 Degradation

Running Title: AKT1 induces β -TrCP-mediated Twist1 degradation

Chia-Wei Li¹, Weiya Xia¹, Longfei Huo¹, Jennifer L. Hsu^{1,7,8}, Yun Wu³, Seung-Oe Lim¹, Chien-Chen Lai⁶, Yi-Hsin Hsu¹, Hui-Lung Sun¹, Jongchan Kim¹, Hirohito Yamaguchi¹, Dung-Fang Lee¹, Hongmei Wang¹, Yan Wang¹, Chao-Kai Chou^{1,7,8}, Jung-Mao Hsu¹, Yun-Ju Lai², Adam M. LaBaff^{1,5}, Qingqing Ding¹, How-Wen Ko^{1,5}, Fuu-Jen Tsai⁶, Chang-Hai Tsai⁶, Gabriel N. Hortobagyi⁴, and Mien-Chie Hung^{1,5,7,8,*}

¹Department of Molecular and Cellular Oncology, ²Department of Experimental Therapeutics, ³Department of Pathology, and ⁴Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

⁵Graduate School of Biomedical Sciences, The University of Texas Health Science Center, Houston, TX 77030, USA

⁶China Medical University, Taichung 404, Taiwan

⁷Center for Molecular Medicine and Graduate Institute of Cancer Biology, China Medical University, Taichung 404, Taiwan

⁸Department of Biotechnology, Asia University, Taichung 413, Taiwan

*Correspondence: Mien-Chie Hung, Department of Molecular and Cellular Oncology, Unit 108, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030. Phone: (713) 792-3668. Fax: (713) 794-3270. E-mail: mhung@mdanderson.org

SUMMARY

Epithelial-to-mesenchyme transition (EMT) is an essential physiological process that promotes cancer cell migration, invasion, and metastasis. Several lines of evidence from both cellular and genetic studies suggest AKT1/PKB α serves as a negative regulator of EMT and breast cancer metastasis while AKT2 and AKT3 generally act as oncogenes to promote tumorigenesis. However, the underlying mechanism by which AKT1 suppresses EMT remains poorly defined. Here, we demonstrate that Twist1 phosphorylated by AKT1 is required for β -TrCP-mediated Twist1 ubiquitination and degradation. The clinically used AKT inhibitor MK-2206, which possesses higher specificity toward AKT1, stabilizes Twist1 and enhances EMT in breast cancer cells. This adverse effect can be overcome by combinatory therapy of MK-2206 and resveratrol to induce β -TrCP mediated Twist1 degradation.

Figure 1

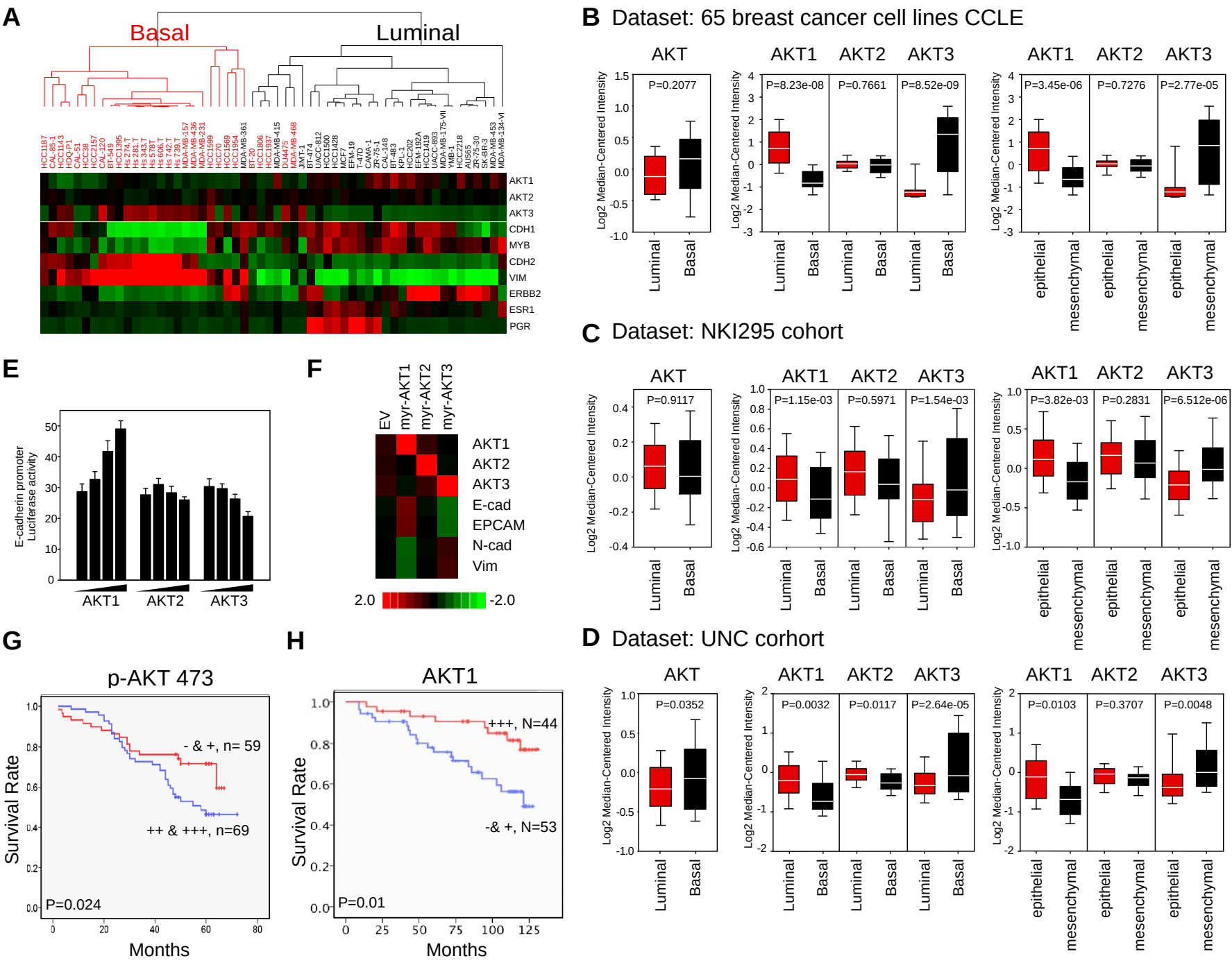


Figure 2

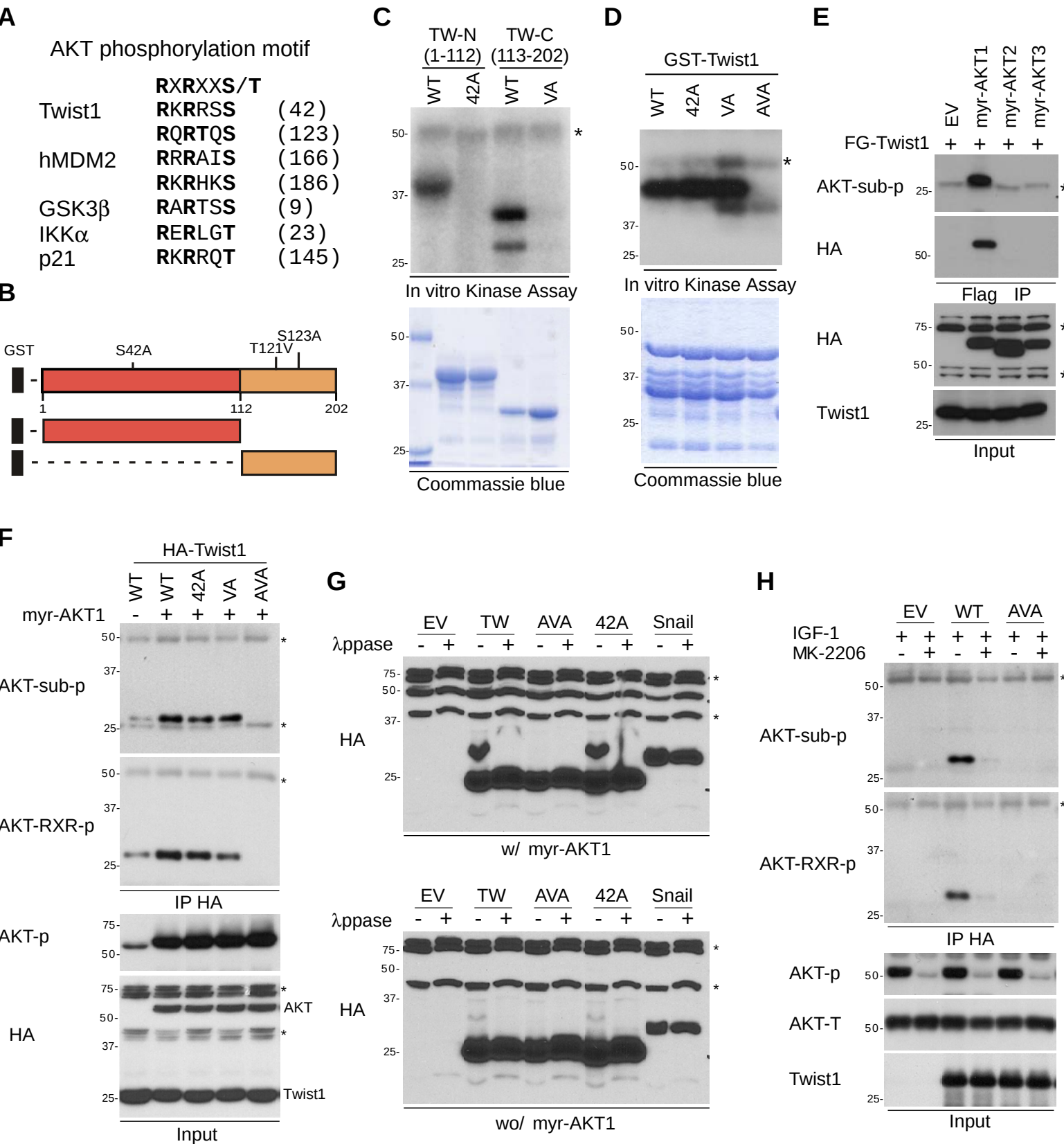


Figure 3

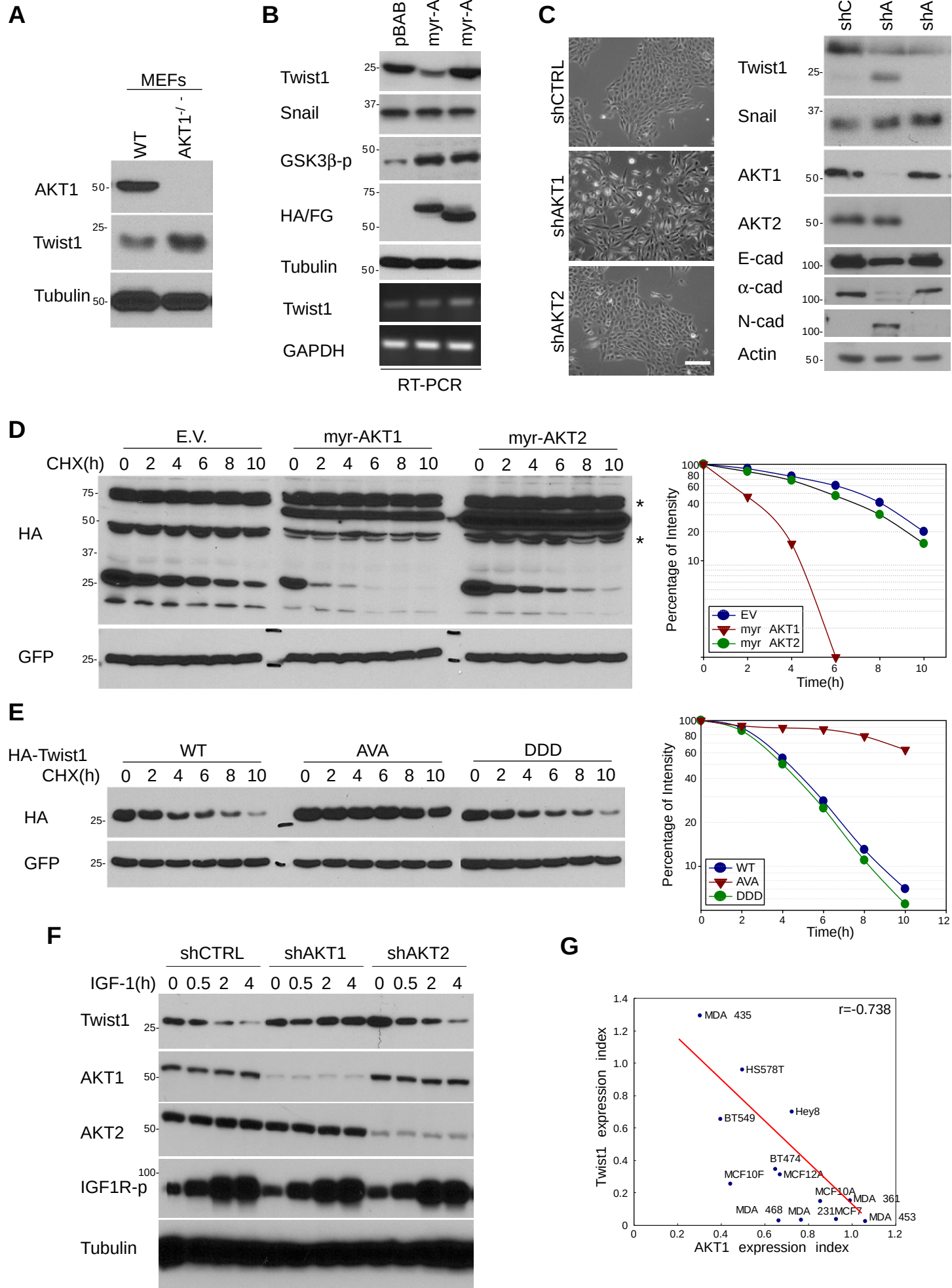


Figure 4

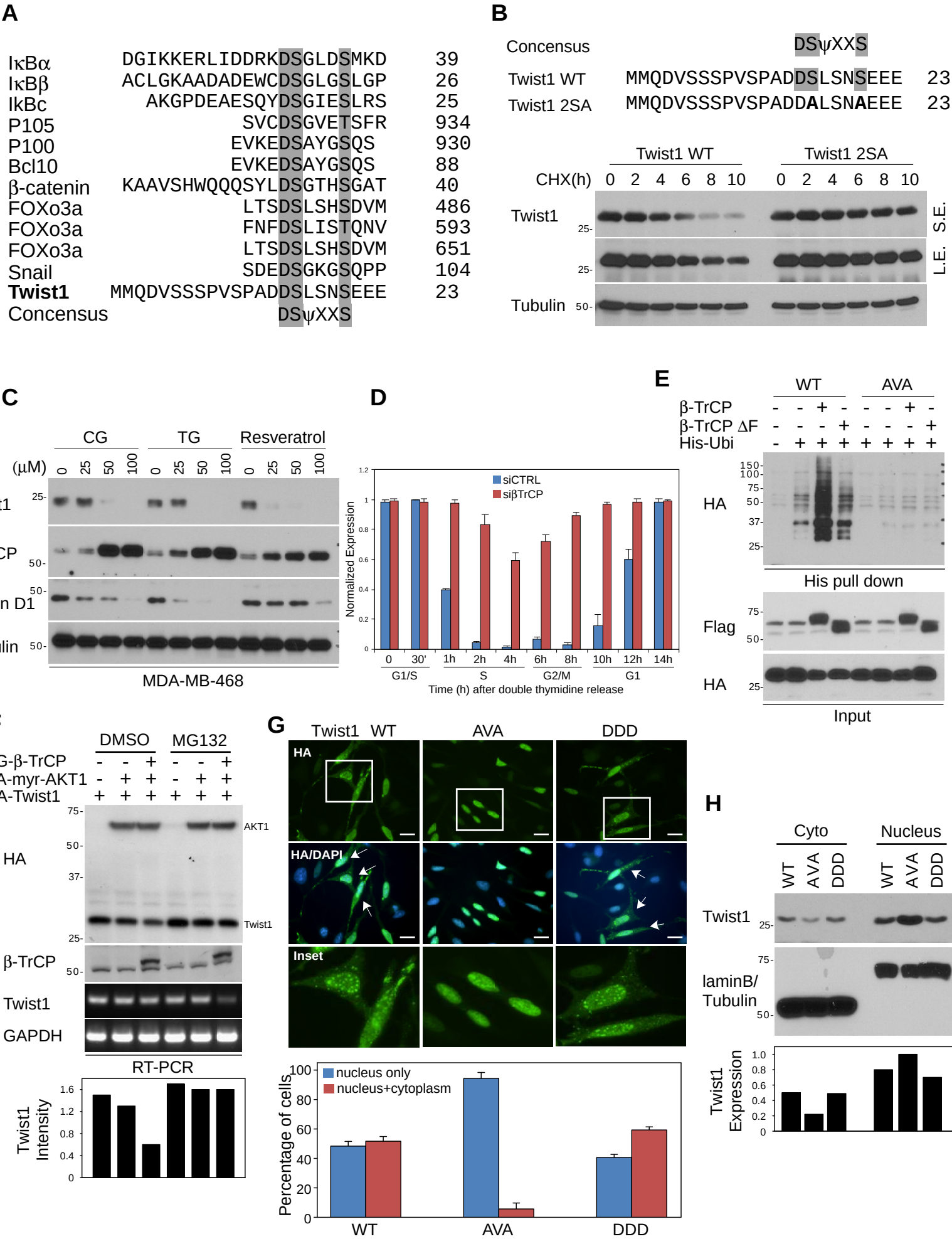


Figure 5

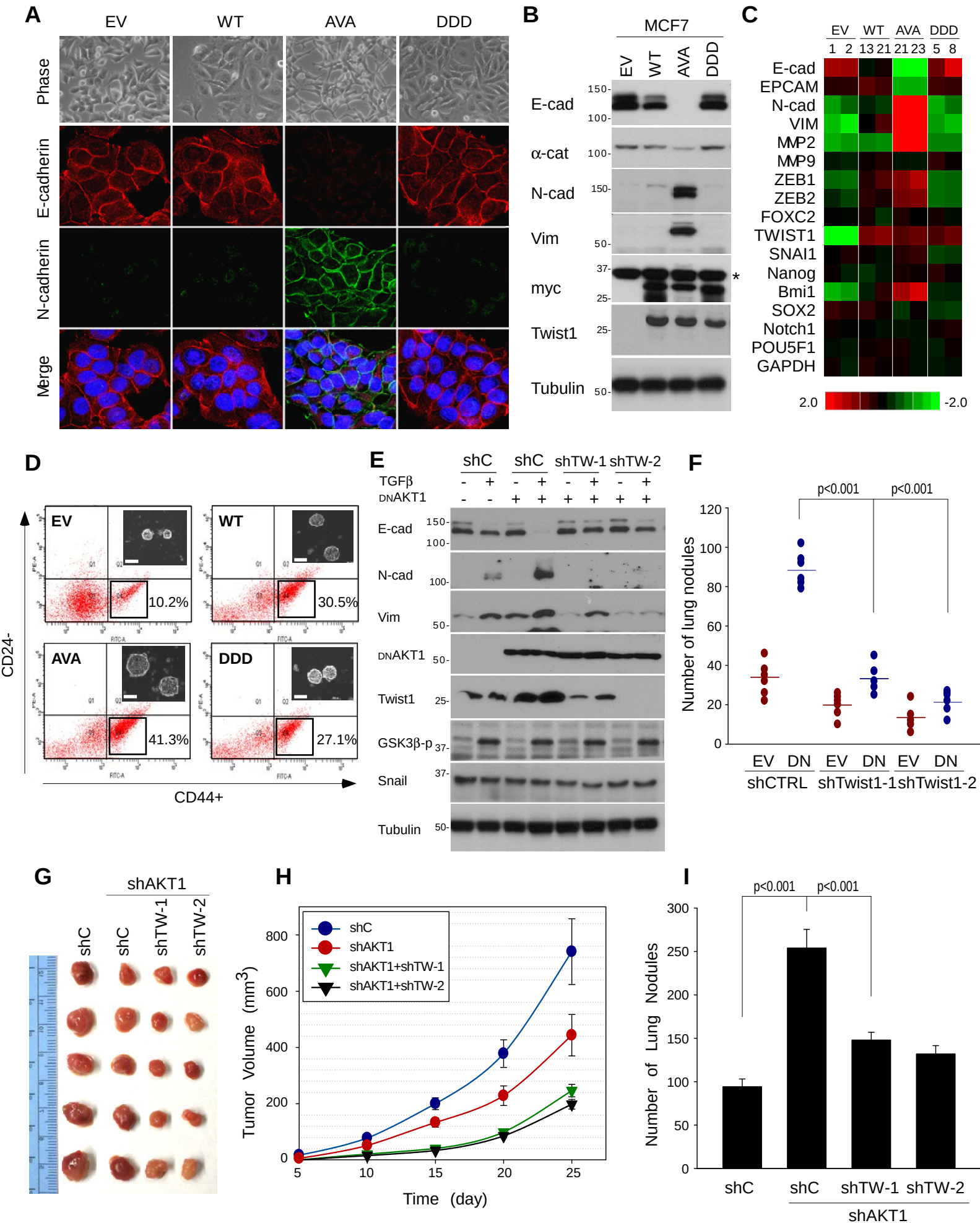


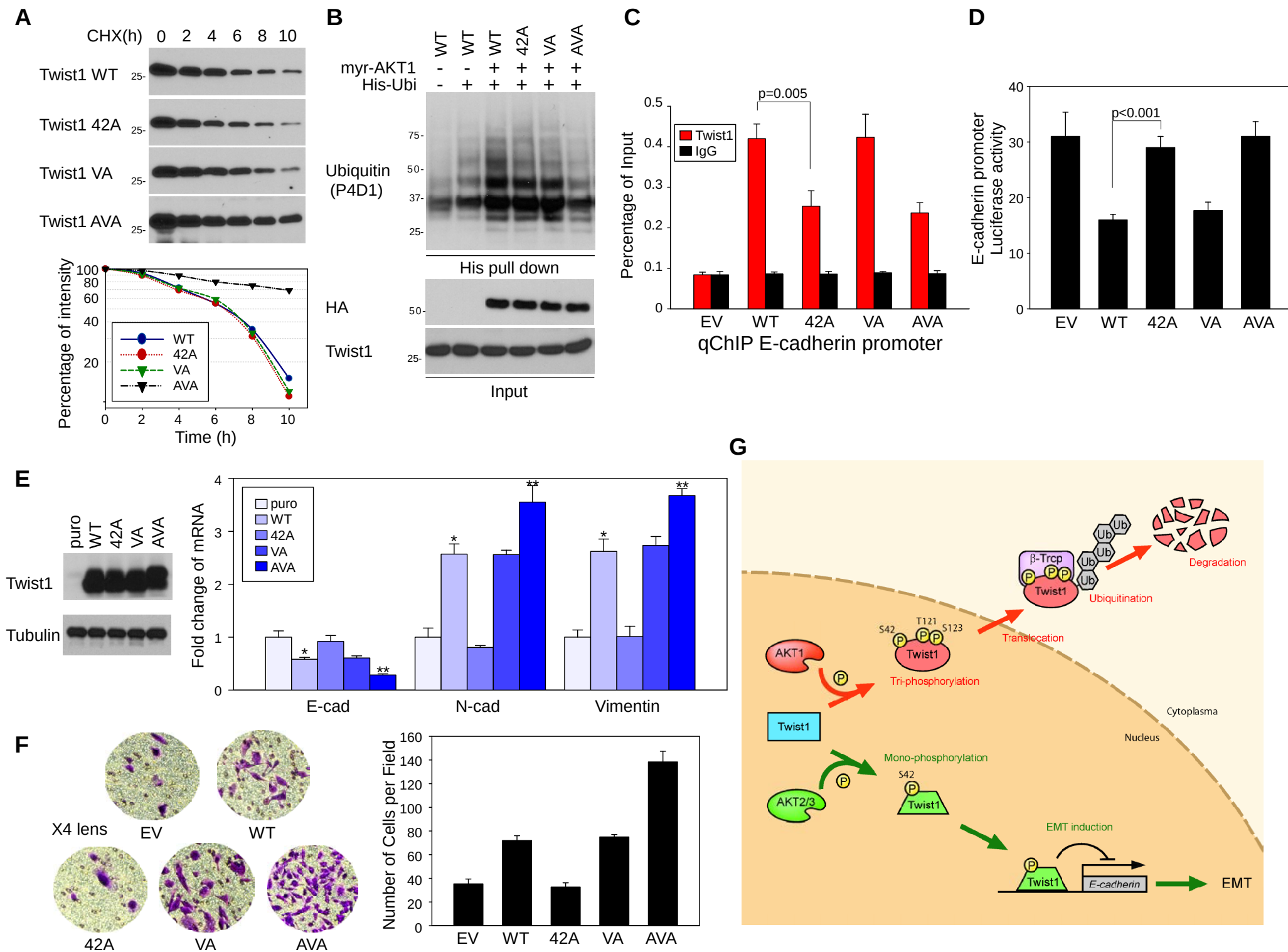
Figure 6

Figure 7

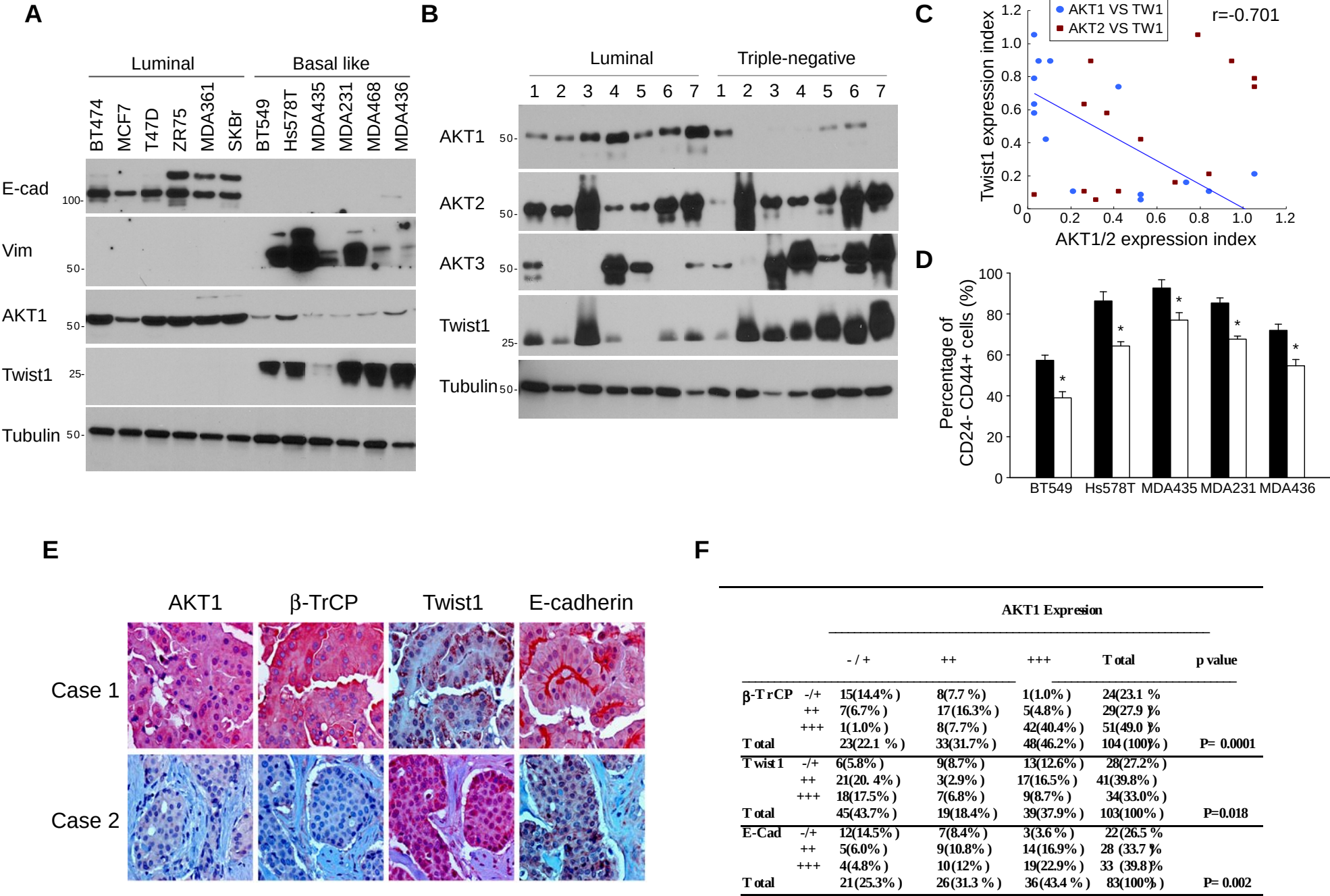


Figure 8

