of the boxes are meant to represent the approximate degree of resulting impairment. For example, deletion of the filamin binding domain resulted in the loss of all functions except growth promotion, which was only partially affected.

SUPPLEMENTARY MATERIALS

Table S1: Sequence of PCR Primers used to quantify mRNAs encoding vWFR subunits in human cells. Location refers to the 5'-end of the primer in the cDNA's according to the GenBank numbering system.

Name of Primer and location Length (bp)	**Sequence (5'→3')**	**Product**
GpIbα (FWD) 781 (GenBank No. NM_000173)	CTG CAG GAC AAT GCT GAA AA	200
GpIbα (REV) 981	GCC CTC AGT GTC CTC TTC TG	
GpIbβ (FWD) 702 (NM_000407)	CCT GCA AAC TCG ACA GGA C	230
GpIbβ (REV) 932	CAG AGT TTG GAA GGG AGA CG	
GpV (FWD) 1749 (L11238)	TGG GTA AAC CAA TGG GAA AA	197
GpV (REV) 1946	GGC CTG TGA GAA TGA AGA GC	
GpIX (FWD) 151 (NM_000174)	AGA AGG CTG AGA CCC GAG A	234
GpIX (REV) 385	ACG GAC TGA AGG CTG TTG TT	

Table S2: Sequences of GpIbα mutagenic primers used for generation of internal deletions. Only the sequences of the "top" oligonucleotides are shown.

Name (5'→3')	**Sequence of mutagenic oligonucleotide (top only)**
Del. Sig.	CAG TGG TCG AGC ATG CCT * CAC CCC ATC TGT GAG GTC
Del. (25-75)	ATC TGT GAG GTC TCC AAA * CTG AAC CTA GAT AGG TGC
Del. LRR/vWF (75-210)	TAC ACT CGC CTC ACT CAG * CAC CTC CTG CCT TTT GCT
Del. Thromb.(271-319)	GAC AAT TCA GAC AAG TTT * ACC CCC TGG GGT CTA TTC
Del.Polym. (405-442)	ATG ACC ACC CTG GAG CCC * ACA AGC CTG ATC ACT CCA
Del. (620–626)	AGC ACA GTG AGC ATT AGG*GTC GAC ACC ATG GCA GAG
Del. (592-627)	TGG GTA CGG CCT AAT GGC*GTC GAC ACC ATG GCA GAG
Del. (567-627)	CAA GTG ACA GTG CCC CGG * GTC GAC ACC ATG GCAGAG
Del Fil. (540-590)	CCA CAG GCC CTG GAC TCT * GGC CGT GTG GGG CCT CTA
C384/385S**	TTT CTC CAC CCC GAC TTT TCC TCC CTC CTC CCC CTG GGC TTC

*indicates the point corresponding to the deleted sequence.
**Cys384/385 (TGT) were mutated to Ser (TCC, underlined).

Table S3: **Function(s) of deleted/mutated regions of GpIbα**

Deletion/Mutant Name	Amino Acids Affected	Function(s) of Deleted Region
Del. Sig.	3-16	Signal peptide; membrane/ER localization
Del. (25-75)	25-75	O-linked glycos. site; contributes to vWF binding
Del. (LRR/vWF)	75-210	Leu-rich domain; vWF binding
Del. Thromb.	271-319	Thrombin binding
Del. Polym.	405-442	13 amino acid domain repeated up to four times/?function
Del. (620-627)	620-627	14-3-3-ζ binding; signaling; regulation of megakaryocyte ploidy
Del. (592-627)	592-627	14-3-3-ζ binding and phosphorylation site
Del. (567-627)	567-627	14-3-3-ζ binding, phosphorylation site, and partial overlap with filamin binding domain
Del. Fil.	540-590	Filamin binding; actin cytoskeleton interaction
C484/485S	C484 & C485	Disulfide binding with GpIbβ; membrane stabilization

For additional information see http://ca.expasy.org/uniprot/PO7359.

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. **Subcellular localization of GpIbα.** *A.*Rat1a1-GpIbα cells and Rat1a-vector cells were fixed in 4% paraformaldehyde-PBS and immunostained with the 9E10 mAb directed against the c-Myc epitope of GpIbα. Secondary staining was with Alexa Fluor 594-conjugated goat anti-mouse IgG as described in Experimental Procedures. B. Staining of HeLa cells as in A except that the anti-GpIbα mAb WM23 was used. C. Staining of HeLa cells as in A except that the LJ-Ibα1 mAb was used. In B and C, the secondary antibody was Alexa Fluor 610-conjugated goat anti-mouse IgG.

Fig. S2. **Ectopically expressed GpIbα fails to co-localize with the TGFβ2 receptor at**

the cell surface. Rat1a cells expressing each of the indicated myc epitope-tagged GpIbα mutants were fixed and stained with a mouse anti-GpIbα mAb (LJ-Ibα1) obtained from Dr. Zaverio Ruggieri Secondary staining was with Alexa-Fluor610-labeled goat anti mouse IgG. Cells were then stained with a rabbit mAb against the cell surface TGFβ2 receptor followed by secondary staining with AlexaFluo488 goat anti rabbit Ig. Confocal microscopy was then performed as previously described (26). Typical fields are shown and depict a striking lack of co-localization of GpIbα (red) and TGFβ2 receptor (green) at the cell surface

Fig. S3. **PI profiles of colcemid-treated Rat1a cell lines.** Cell lines expressing each of the mutants depicted in Fig. 2 were treated with colcemid (25 ng/ml, Sigma-Aldrich, St. Louis, MO) for 16 hr and then stained with PI. Cell cycle analyses were performed on propidium iodide-stained nuclei using a FACSstar flow cytometer (Becton-Dickinson Biosciences, San Jose, CA). Data were analyzed by single histogram statistics as previously described (13,14) and were gathered from at least three experiments similar to those depicted here.

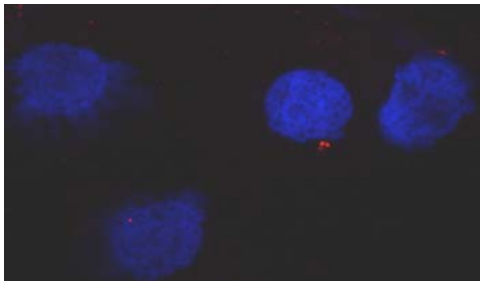
Fig. S4. **Composite images of typical nuclei following exposure of Rat1a cells with DCB.** Control cells or those expressing the indicated mutants were grown in the presence of 2 μM DCB for 24 hr, fixed in 4% paraformaldehyde/PBS, and stained with DAPI. Note the presence of 2 nuclei of equal size and shape in virtually all Rat1a-vector (control) cells. In contrast, note the large number of cells with >2 nuclei, as well as size and shape discrepancies and other dysmorphic features in Rat1a-GpIbα cells. See Fig. 5B for quantification of at least 200 cells expressing each of the mutants.

Fig. S5. **γ-H2AX foci in Rat1a cells.** Cells from the indicated groups were plated onto glass coverslips and allowed to attain log-phase growth (1-2 d) before fixing in 4% paraformaldehyde-PBS. Coverslips were then stained with an antibody specific for Ser139 of histone H2A (γ-H2AX) (17). Note the absence of any significant staining of Rat1a-vector cells in contrast to the large percentage of such cells in the Rat1a-GpIbα group. Although significant differences in the percentage of positive cells was seen among the various Rat1a groups, the appearances of the γ-H2AX foci among the positive cells in these groups were indistinguishable. See Fig. 5C for quantification of at least 200 cells expressing each of the mutants.

A

Gplba/DAPI

Rat1a-vector



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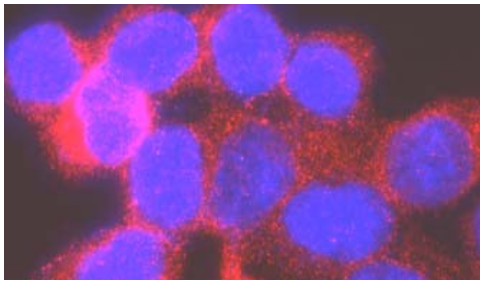
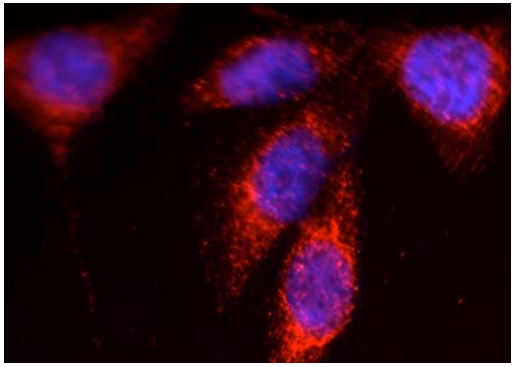
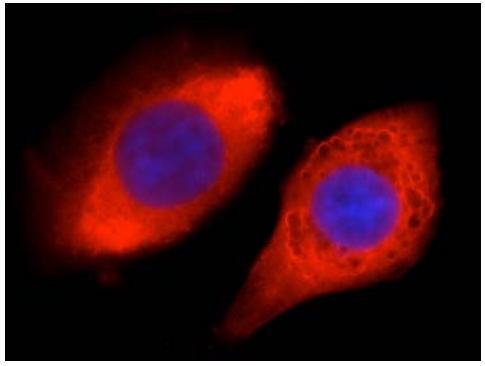


B



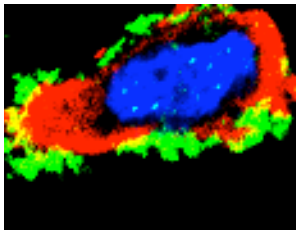
WM23

C



LJ-Ibα1

Gplbα/TGF-β-R2



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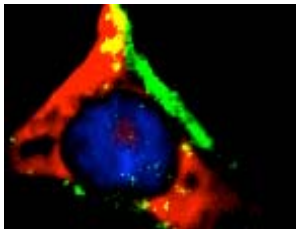

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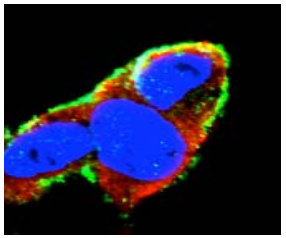

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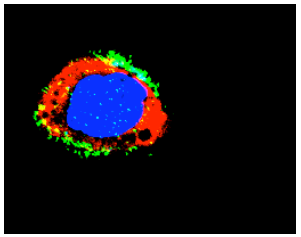

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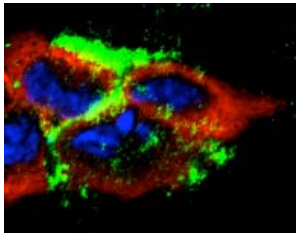

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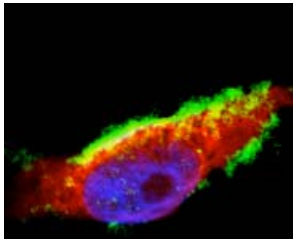
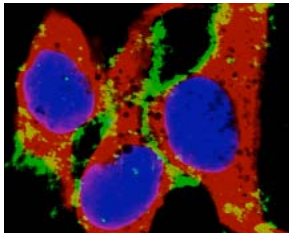


Del polym



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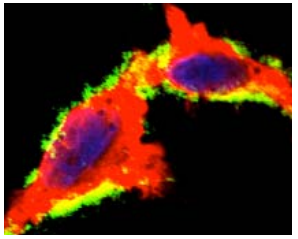

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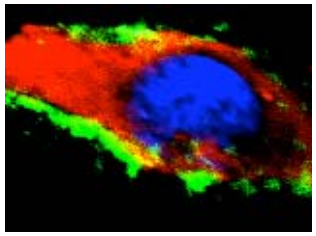

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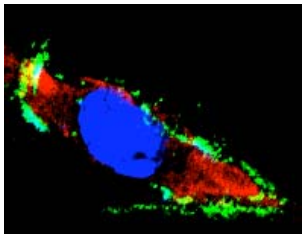

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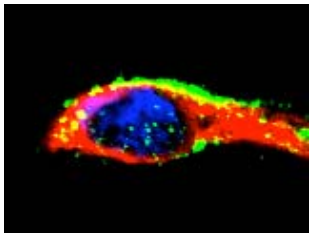

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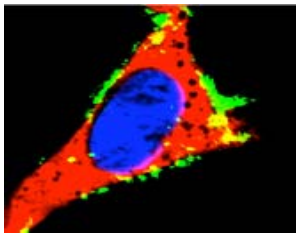
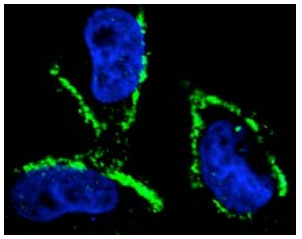


C484/485S



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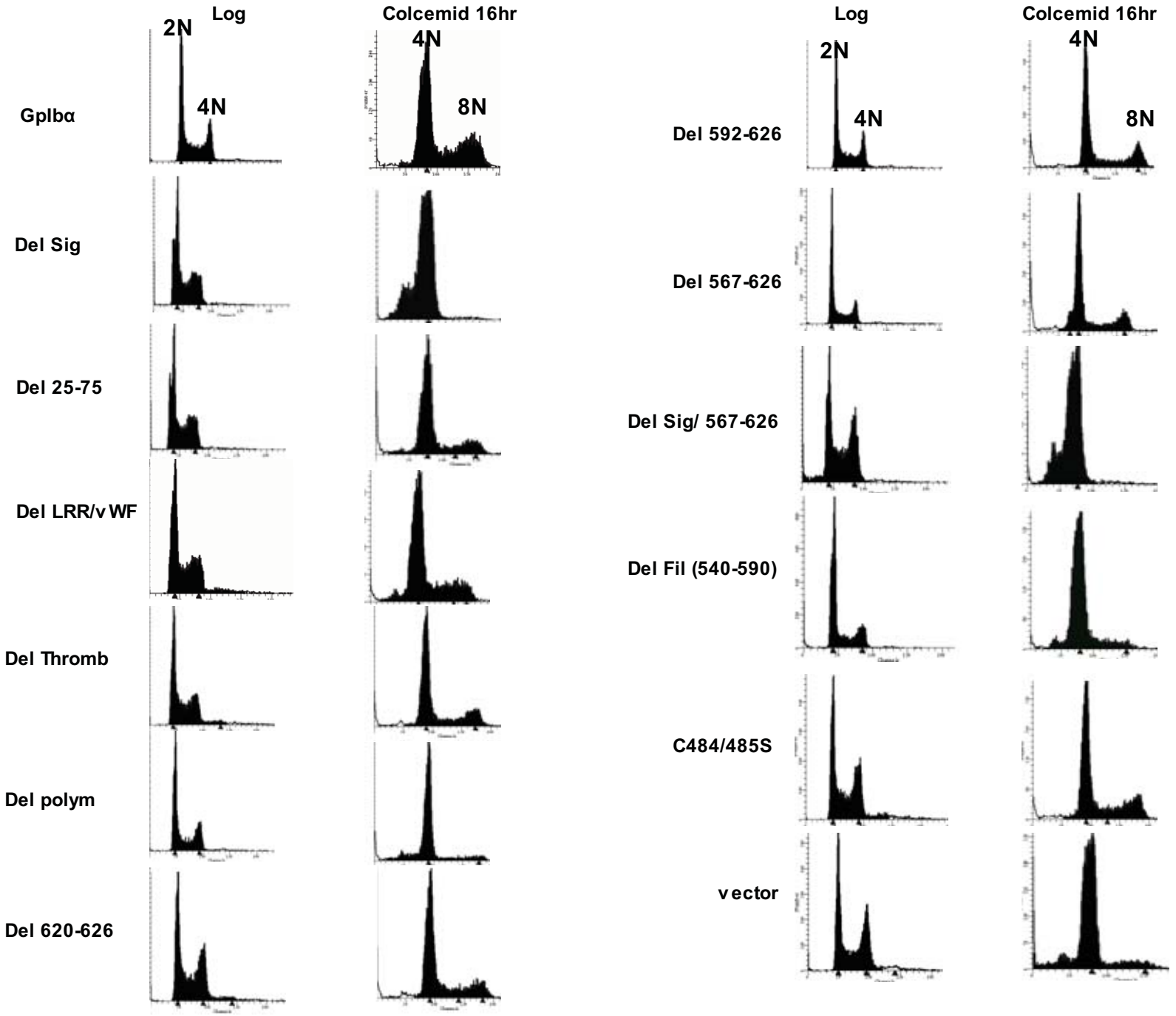
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8N
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Del 620-626
Log
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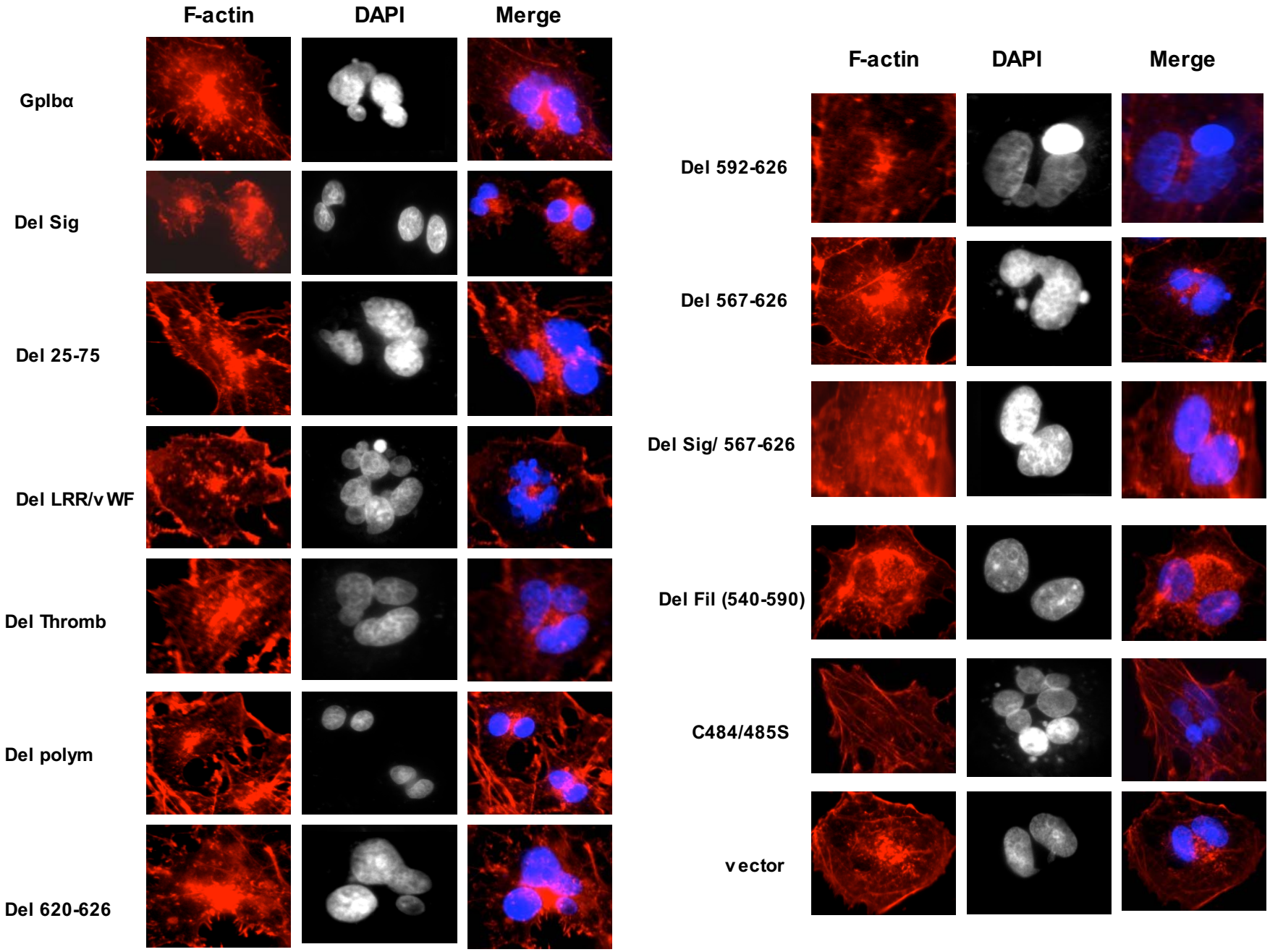
Del Fil (540-590)
C484/485S
vector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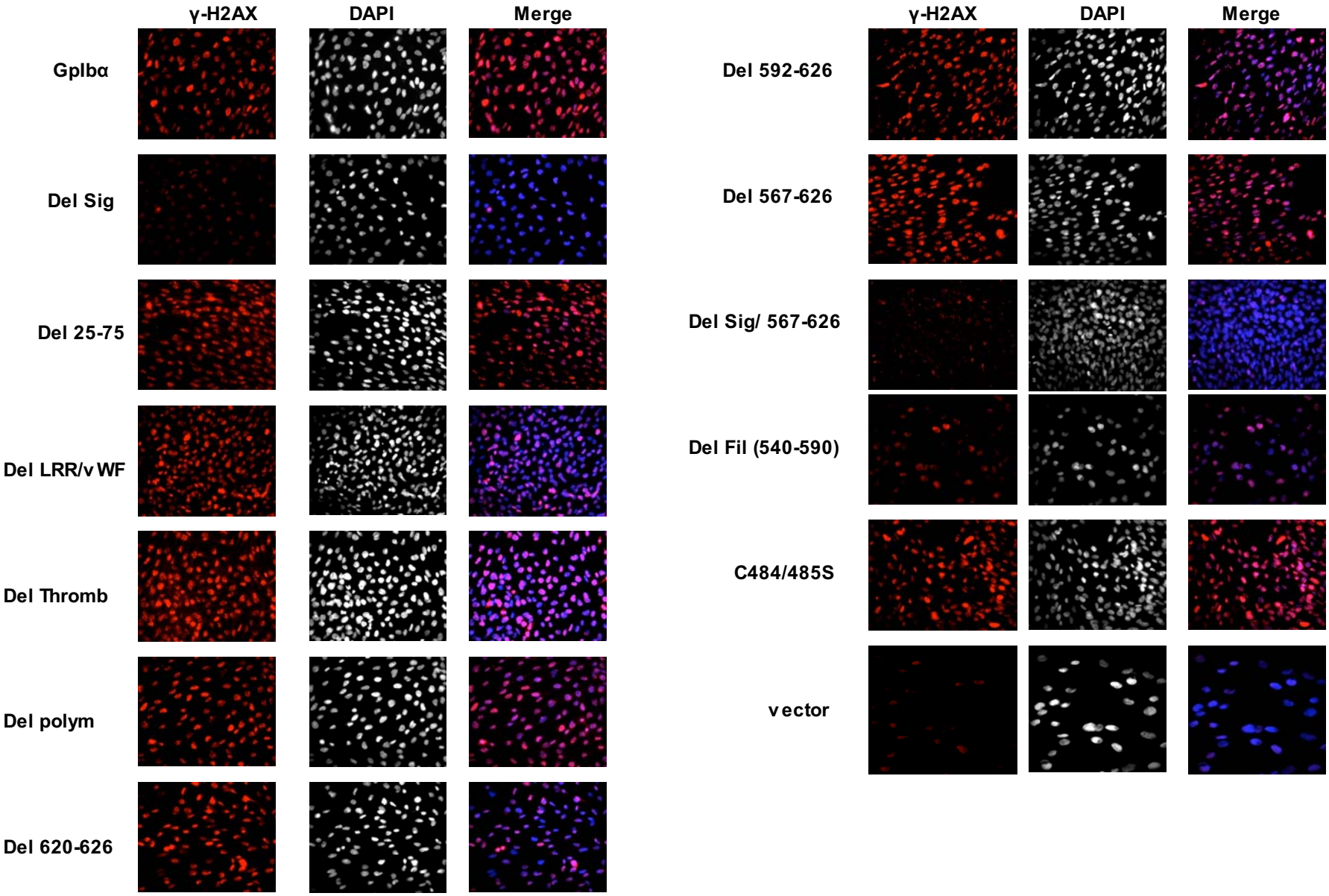
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