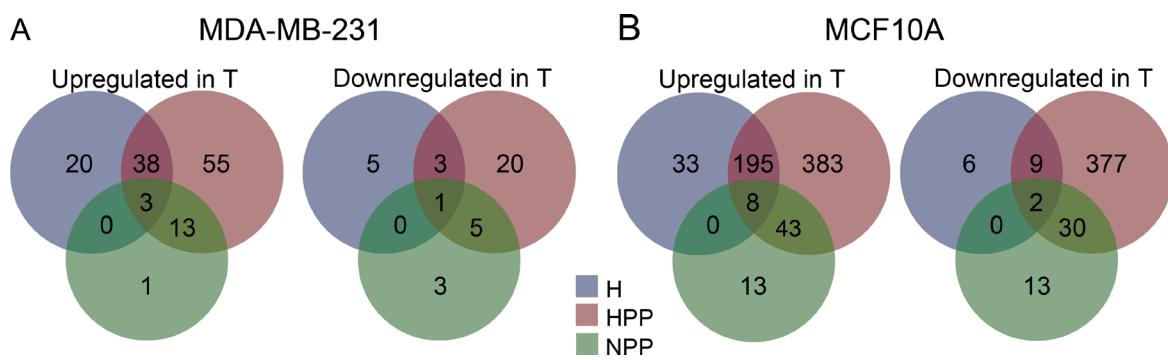
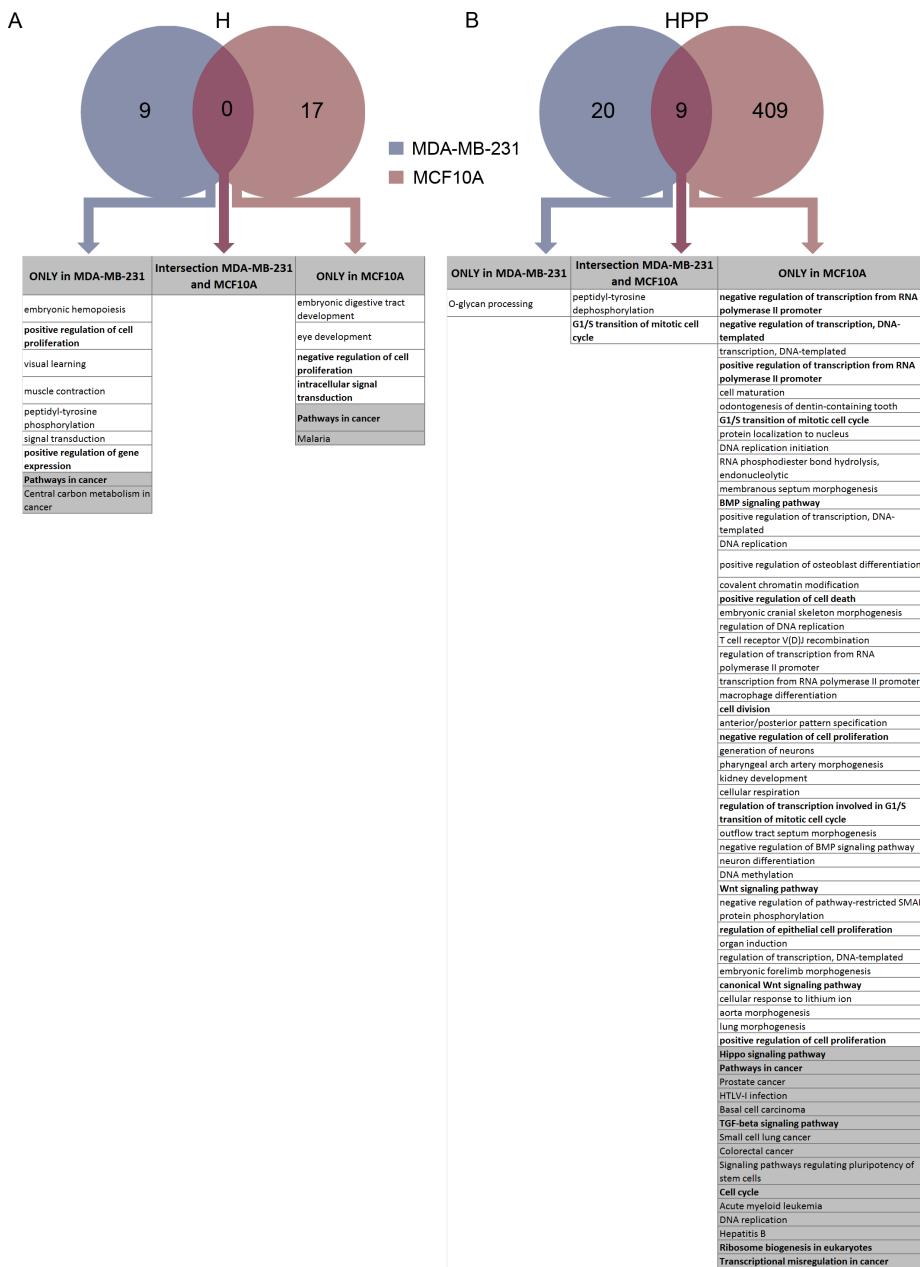


## Hypoxia-mediated translational activation of ITGB3 in breast cancer cells enhances TGF- $\beta$ signaling and malignant features *in vitro* and *in vivo*

### SUPPLEMENTARY MATERIALS



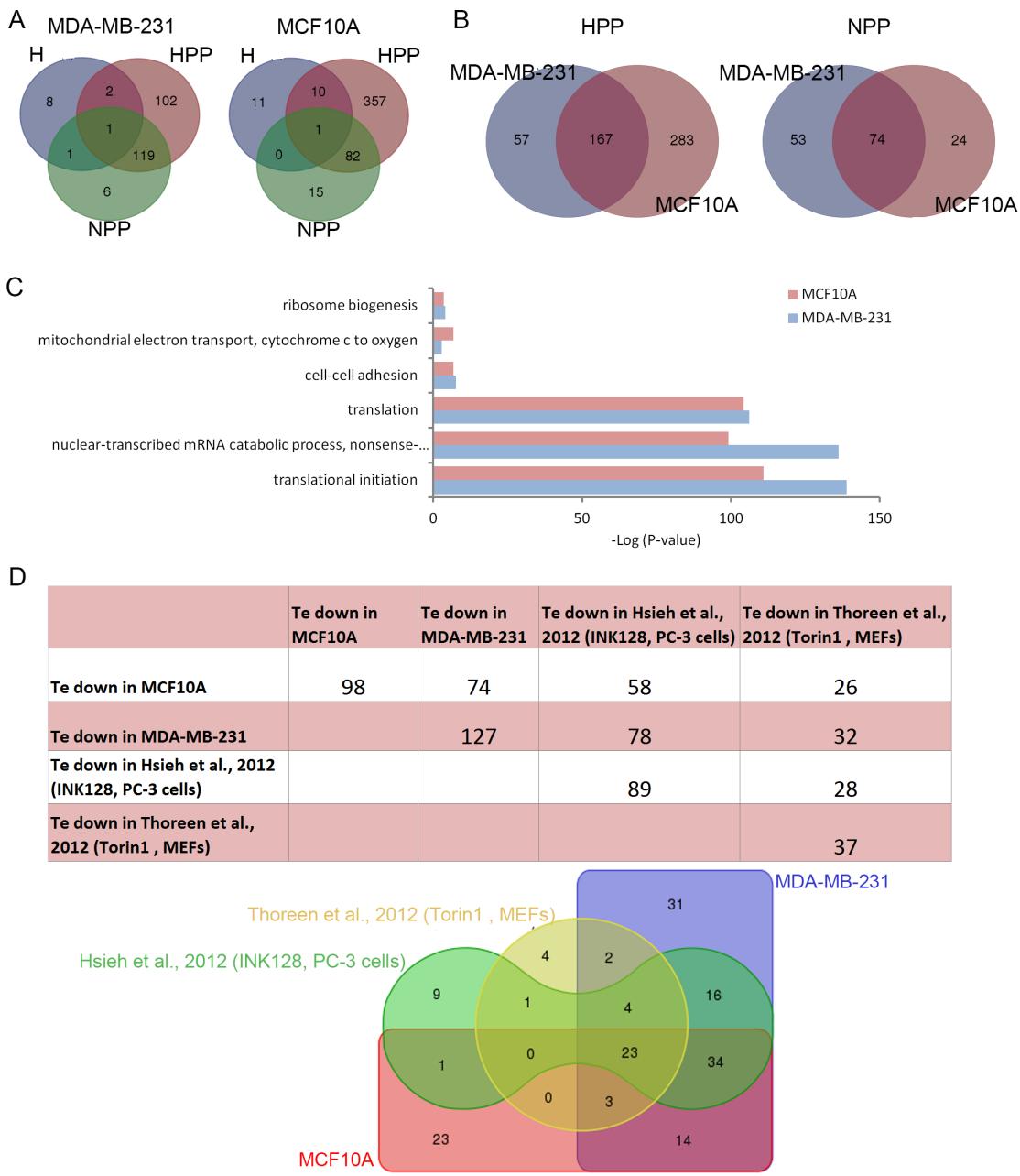
**Supplementary Figure 1:** Venn diagrams showing the distribution of transcriptionally deregulated genes in H, HPP and NPP in MDA-MB-231 (**A**) and MCF10A (**B**) cell lines.



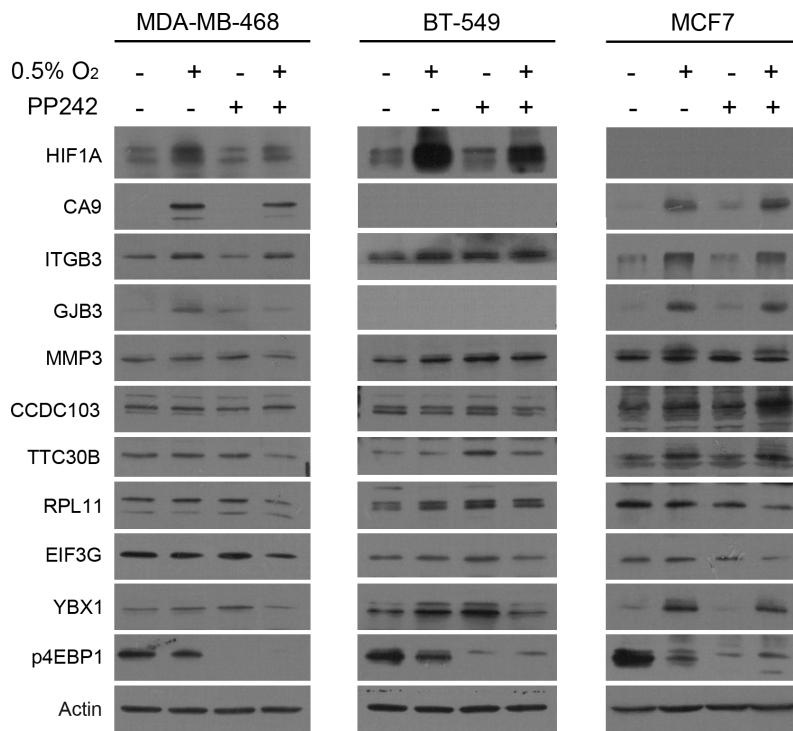
**Supplementary Figure 2: Analysis of transcriptionally downregulated genes under hypoxic and hypoxic + PP242 conditions.** (A) Venn diagram with the distribution of transcripts downregulated in hypoxia. A table showing the main GO categories (with  $P < 0.03$ ) and Kegg pathways (with  $P < 0.03$ ) associated with each cell line. (B) Venn diagram with the distribution of transcripts downregulated in hypoxia + PP242. A table showing the main GO categories (with  $P < 0.03$ ) and Kegg pathways (with  $P < 0.03$ ) associated with each cell line.



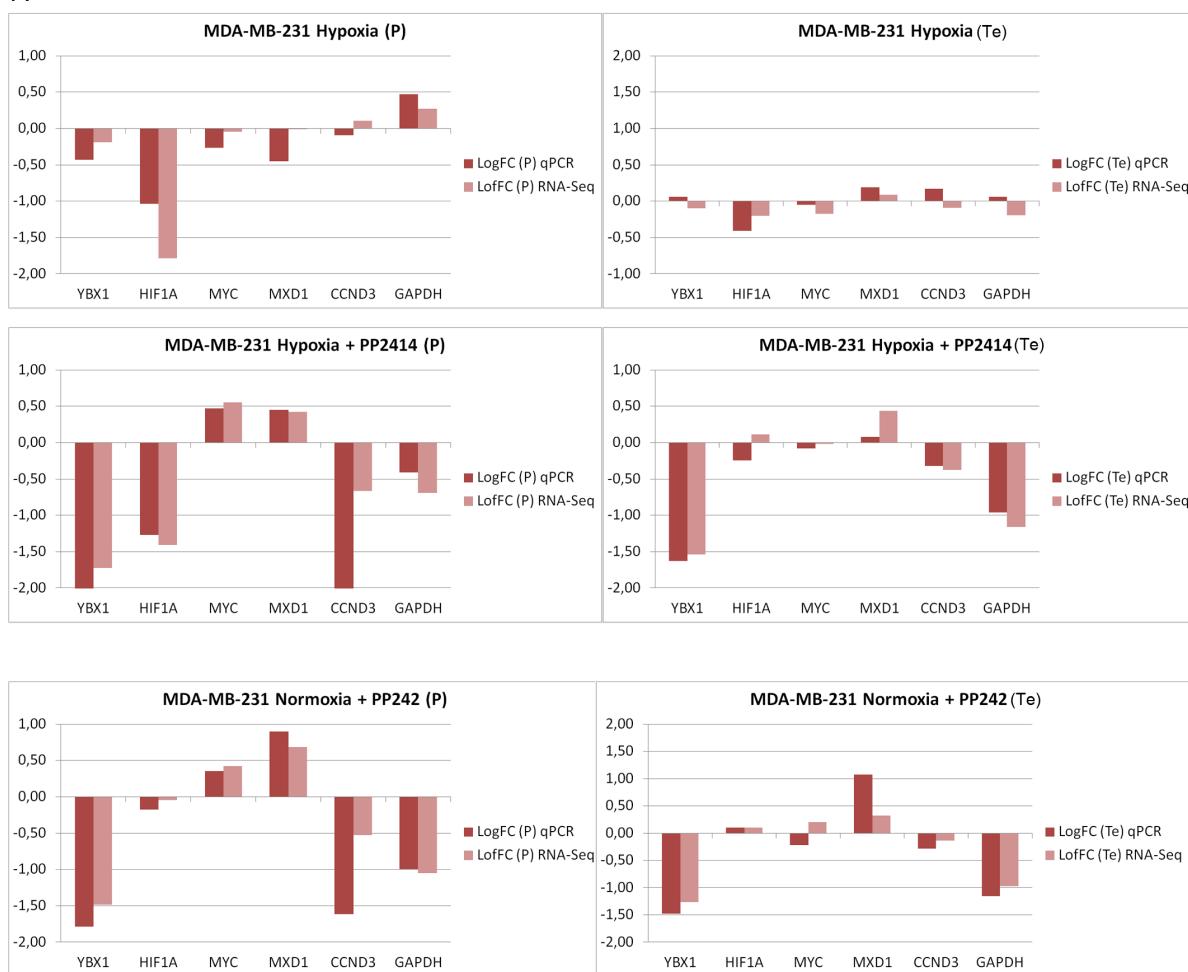
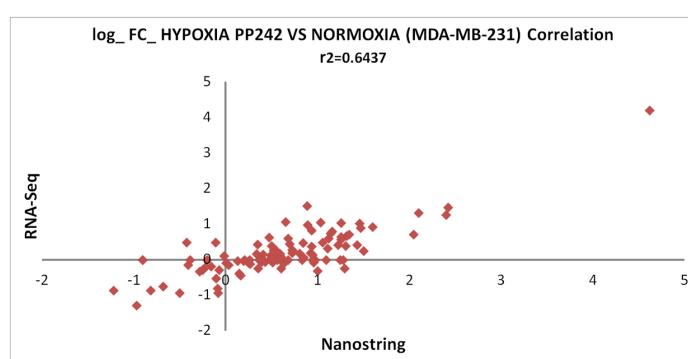
**Supplementary Figure 3: Analysis of transcriptionally deregulated genes when cells are treated with PP242.** (A) Venn diagram with the distribution of transcripts upregulated in NPP. A table showing the main GO categories (with  $P < 0.03$ ) associated with each cell line and the GO categories (with  $P < 0.03$ ) associated with genes in the intersection between the two cell lines. (B) Venn diagrams with the distribution of transcripts downregulated in NPP. A table showing the main GO categories (with  $P < 0.03$ ) associated with each cell line and the GO categories (with  $P < 0.03$ ) associated with genes in the intersection between the two cell lines.



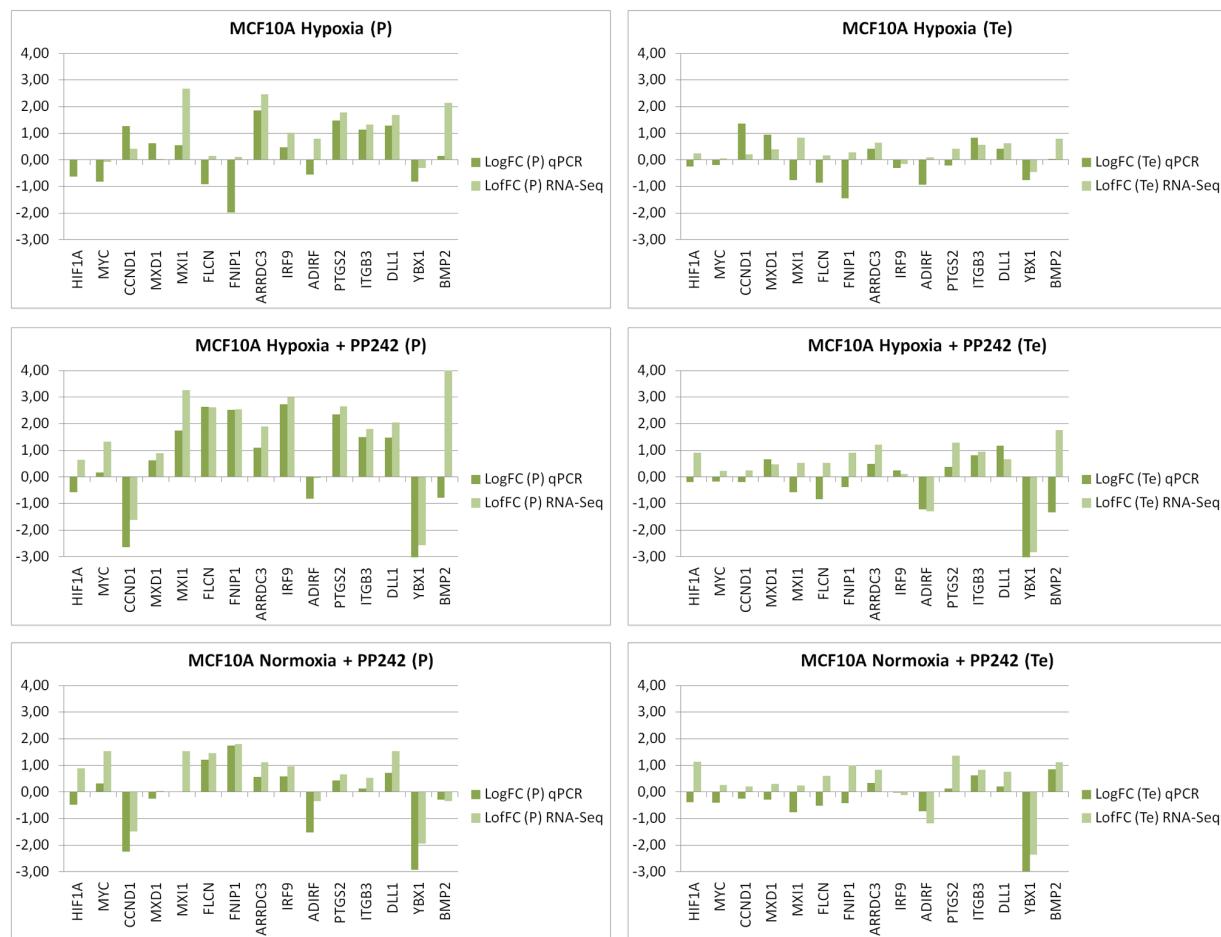
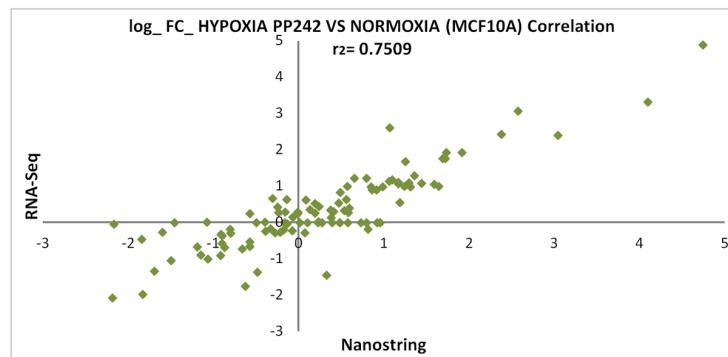
**Supplementary Figure 4: Translationally downregulated transcripts upon PP242 treatment.** (A) Venn diagrams showing the distribution of downregulated transcripts at the protein synthesis level under H, HPP and NPP conditions in each cell line. (B) Venn diagrams showing translationally downregulated transcripts in HPP and NPP highlighting genes in the intersection between the two cell lines. (C) Main GO categories associated with translationally downregulated genes in HPP in both cell lines. (D) Above: Table showing the number of genes translationally downregulated in our study and in others studies. Below: Venn diagram showing the number of genes translationally inactivated in NPP compared with the ones inactivated in other published studies.



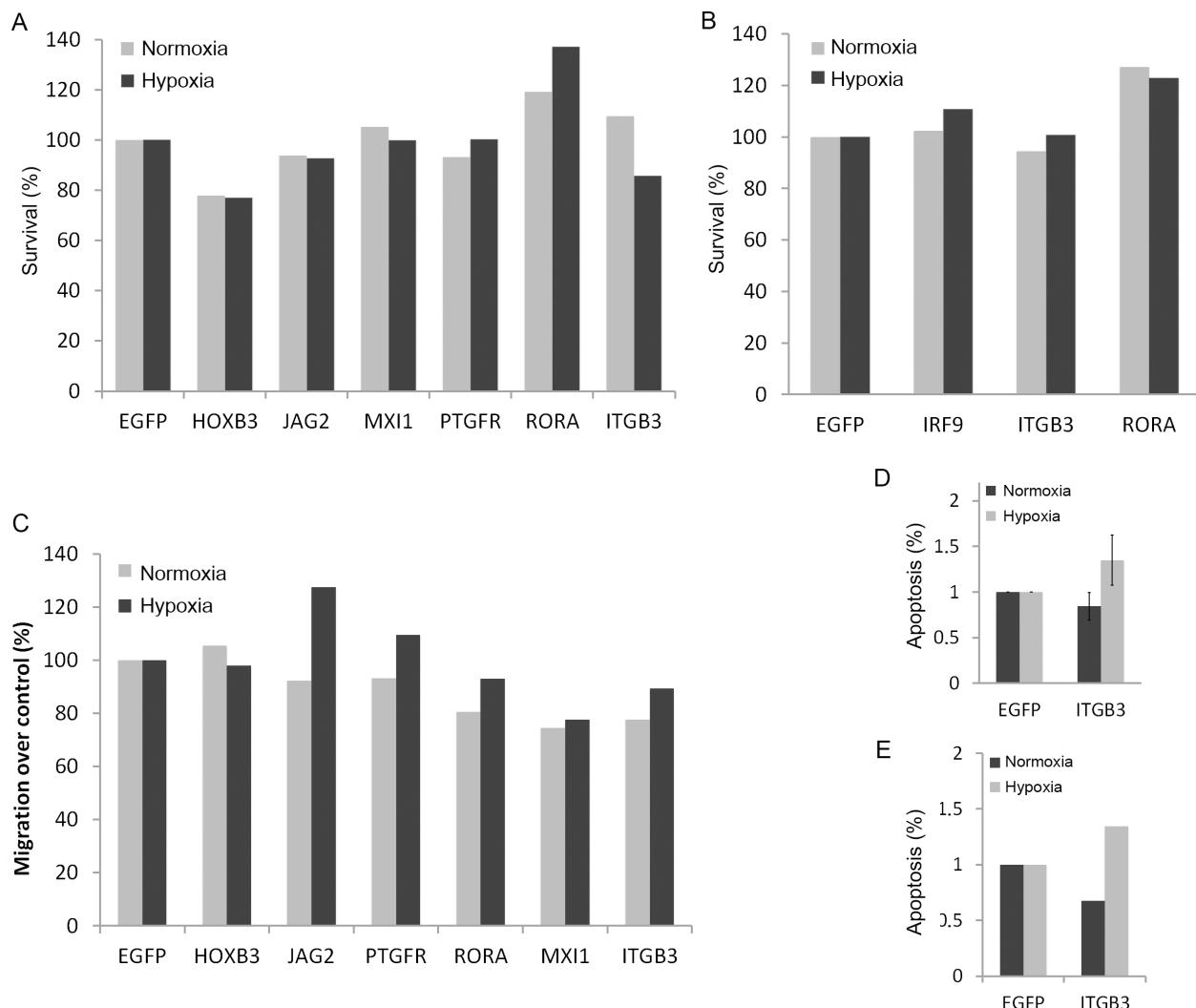
**Supplementary Figure 5: Increased translational efficiency (Te) of ITGB3 is accompanied by increased protein in other breast cancer cell lines.** Immunoblots for all experimental conditions of the different translationally activated (ITGB3, GJB3, MMP3, CCDC103, TTC30B) or inactivated (RPL11, EIF3G, YBX1) targets in HPP in MDA-MB-468, BT-549 and MCF7.

**A****B**

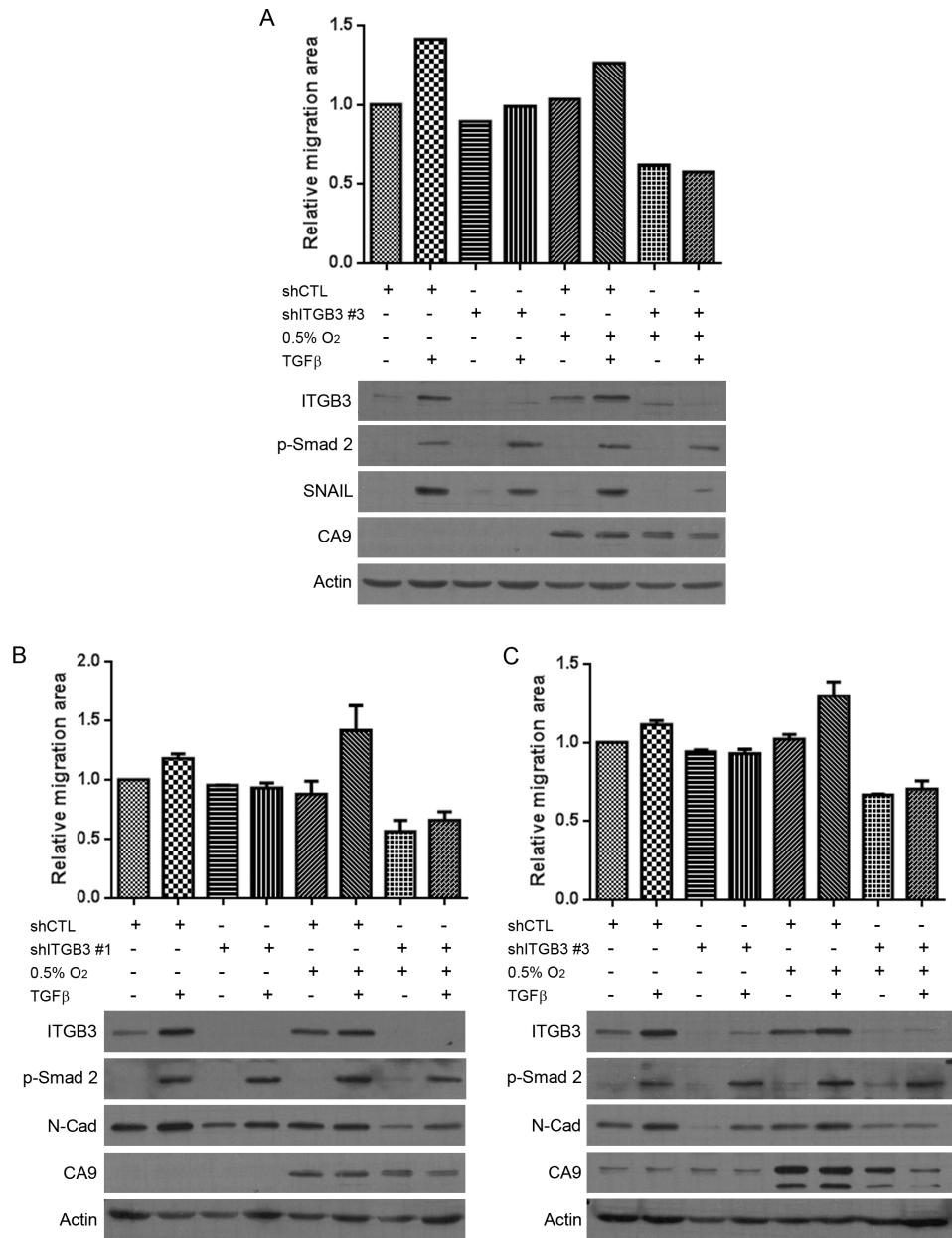
**Supplementary Figure 6: Validation of the screening by qRT-PCR and NanoString in MDA-MB-231 cells.** (A) qRT-PCR of candidate genes in polysomal mRNA (left panels) and the ratio of Log<sub>2</sub>FC P to Log<sub>2</sub>FC T (right panels) compared with the values obtained from the RNA-Seq screening under H, HPP and NPP conditions. (B) Correlation between the 50-gene PAM50 assay and the expression obtained in RNA-Seq for these genes from the total mRNA.

**A****B**

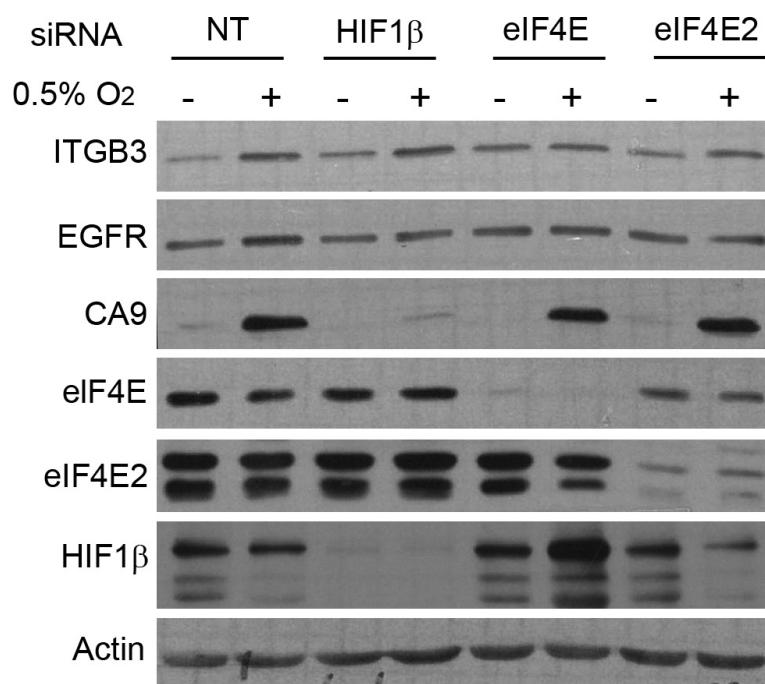
**Supplementary Figure 7: Validation of the screening by qRT-PCR and NanoString in MCF10A cells.** (A) qRT-PCR of candidate genes in polysomal mRNA (left panels) and the ratio of  $\text{Log}_2\text{FC P}$  to  $\text{Log}_2\text{FC T}$  (right panels) compared with the values obtained from the RNA-Seq screening under H, NPP and HPP conditions. (B) Correlation between the 50-gene PAM50 assay and the expression obtained in the RNA-Seq for these genes from the total mRNA.



**Supplementary Figure 8: Screening by siRNA of candidate translationally activated genes in hypoxia with PP242.** (A) Forty-eight hour survival rates in hypoxia compared with normoxia. (B) Forty-eight hour survival rates in hypoxia compared with normoxia in MCF10A cells transfected with the different siRNAs. (C) Migration assays in MDA-MB-231 cells transfected with the different siRNAs and subjected to hypoxia versus normoxia for 8 hours. (D) Apoptosis in MDA-MB-231 cells transfected with the different siRNAs. (E) Apoptosis in MCF10A cells transfected with the different siRNAs.



**Supplementary Figure 9: Migration assay of MDA-MB-231 and MCF10A cells with ITGB3 silencing and treated with TGB- $\beta$ .** (A) Migration assay of MDA-MB-231 cells using shITGB3 #3 (above) and an immunoblot showing ITGB3 and Snail expression upon treatments (below). (B) Migration assay of MCF10A cells using shITGB3 #1 (above) and an immunoblot showing ITGB3 and N-cadherin expression upon treatments (below). (C) Migration assay of MCF10A cells using shITGB3 #3 (above) and an immunoblot showing ITGB3 and N-cadherin expression upon treatments (below).



**Supplementary Figure 10: eIF4E is essential for protein synthesis activation of ITGB3 under low-oxygen conditions.**  
 Immunoblot showing ITGB3 expression under hypoxic conditions after eIF4E2, eIF4E or HIF1 $\beta$  silencing in the MCF10A cell line.

**Supplementary Table 1: Lists of mRNAs that are translationally regulated in MCF10A and MDA-MB-231 cells in all experimental conditions.** See Supplementary\_Table\_1