

**Overexpression of  $\beta$ -Arrestins inhibits proliferation and motility in triple negative breast cancer cells.**

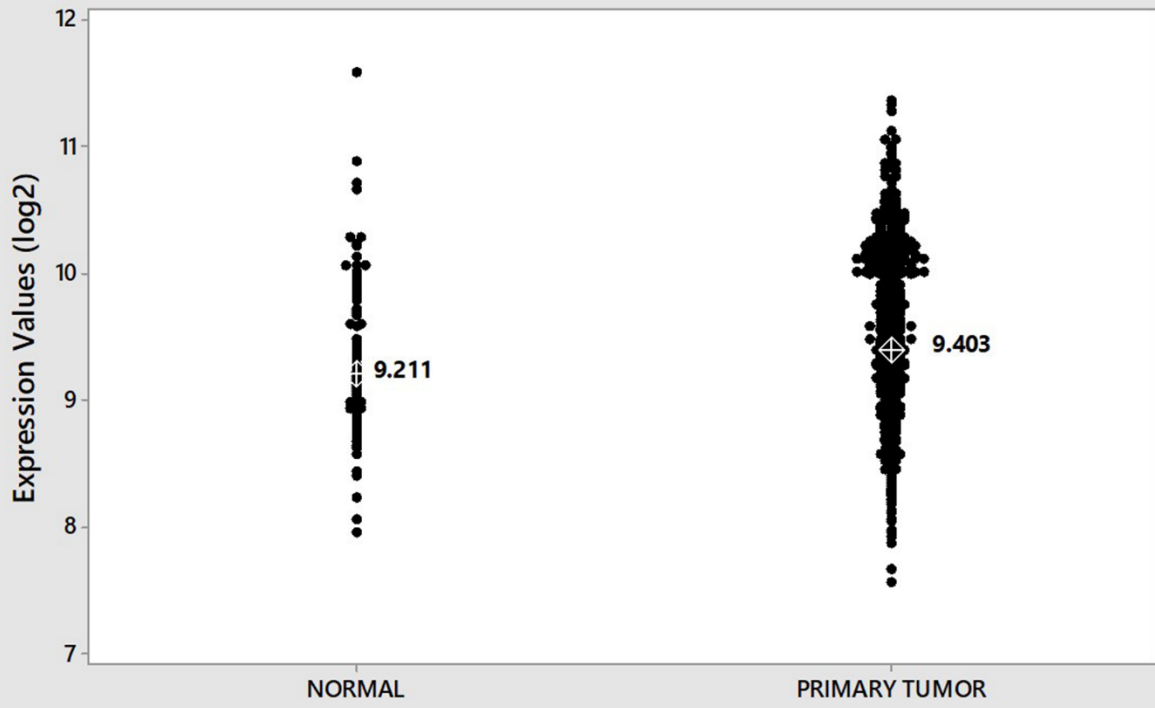
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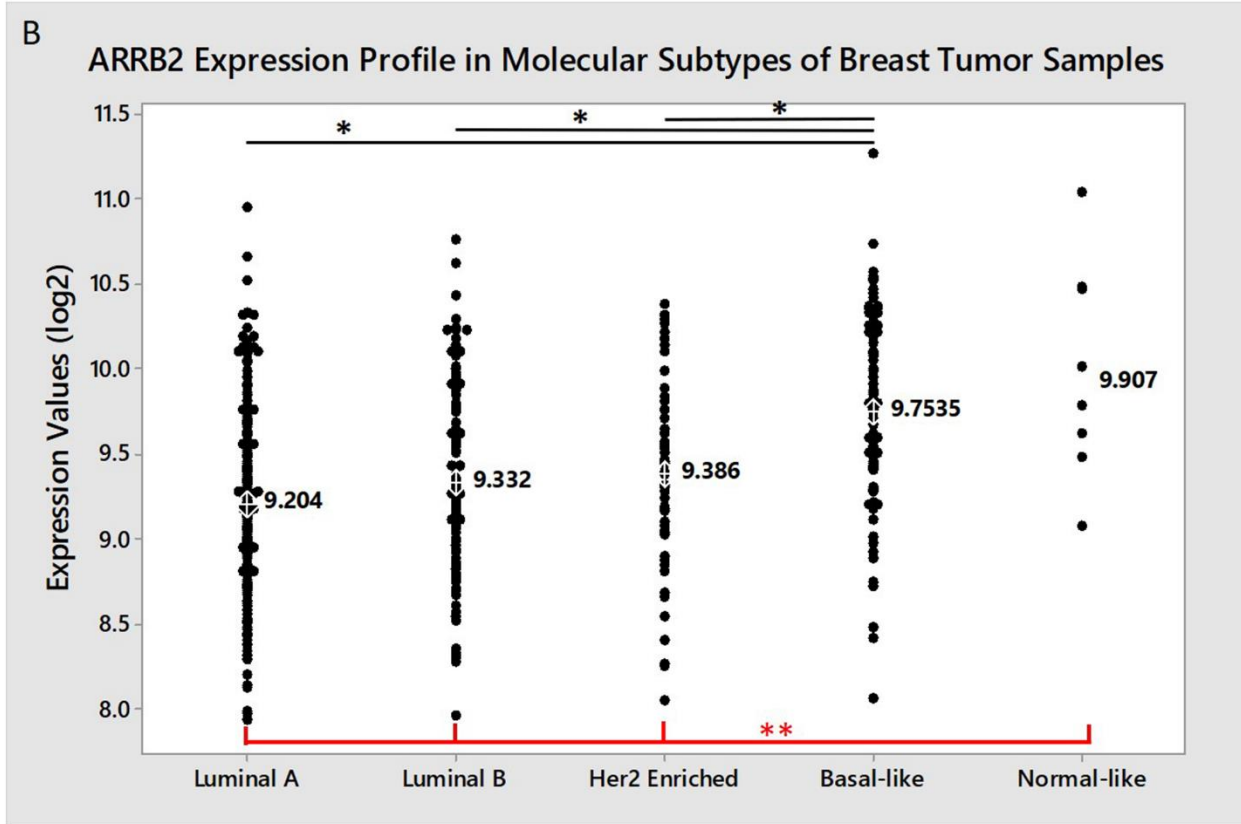
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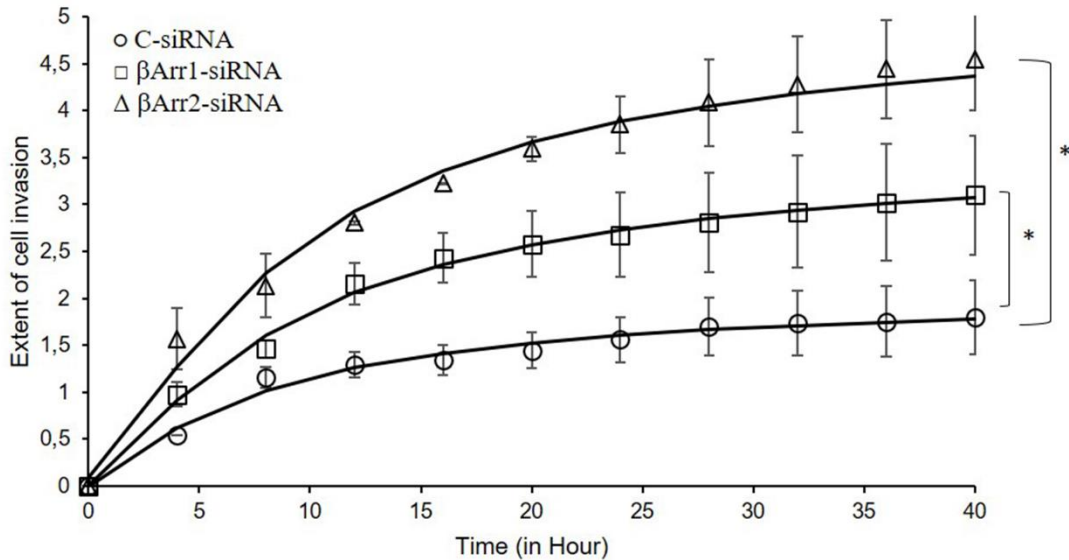
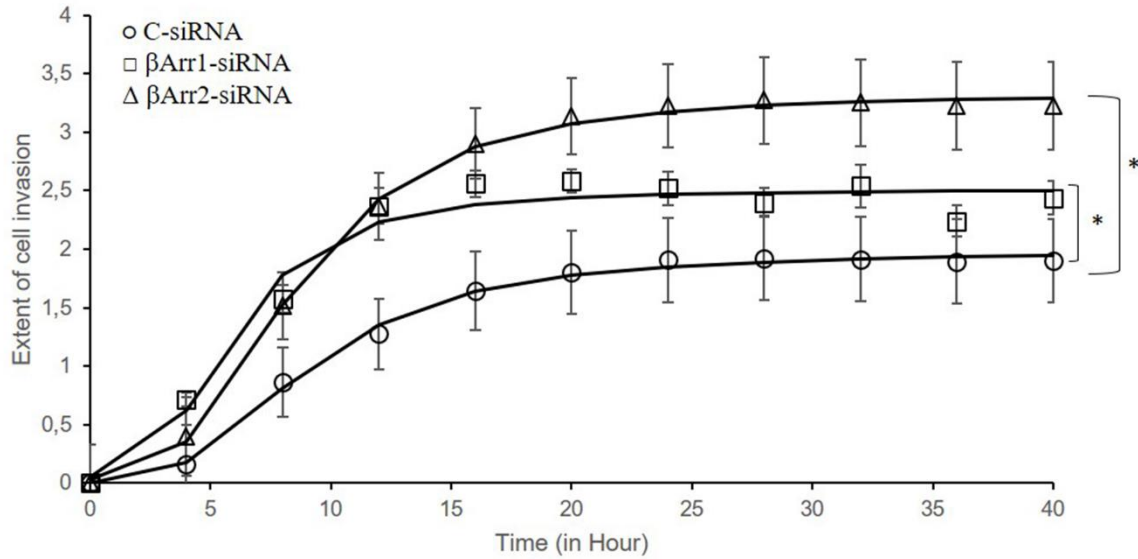
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### ARRB2 Expression Profile in Normal and Primary Tumor Samples



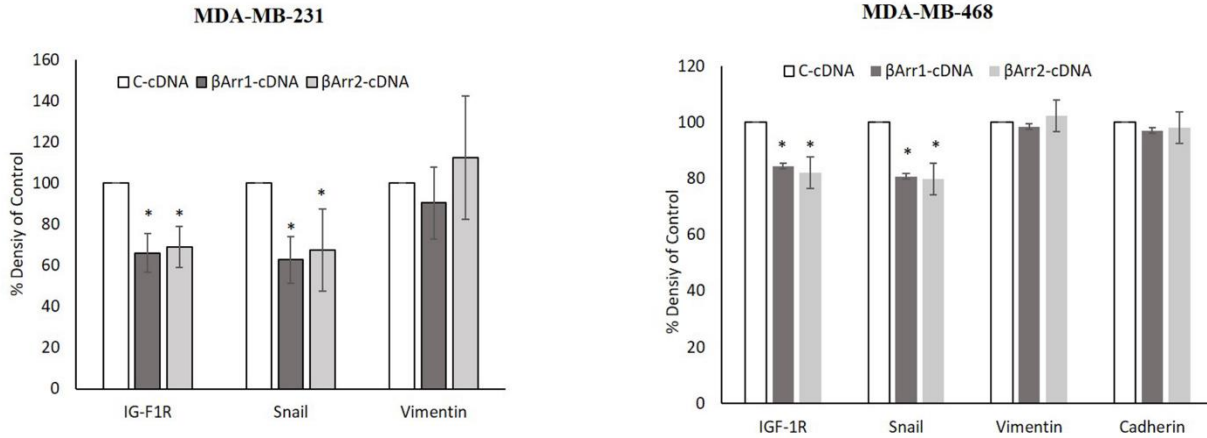


**Supplementary Figure 1. TCGA data analysis results for  $\beta$ Arr2.** A) The expression profiles of  $\beta$ Arr2 (ARRB2) gene in 114 normal samples and 1097 primary tumour samples obtained from TCGA are shown in individual value plots. The median expression values are marked for each sample type, which is 9.211 for normal samples and 9.403 for the tumour samples. ARRB2 gene is significantly upregulated in tumour samples compared to normal samples (t-test;  $p=0.027$ ). B) The expression profiles of ARRB2 gene in 98 basal-like, 58 HER2-enriched, 127 luminal B, 231 luminal A and 8 normal-like breast tumour samples obtained from TCGA are shown in individual value plots (The median values for each sample type are given on the value plots). According to ANOVA results ARRB2 expression is downregulated significantly in Luminal A, Luminal B or Her2 Enriched compared to the Normal-like or Basal-like breast tumours (ANOVA;  $*p<0.0001$ ,  $**p<0.005$ ). ARRB2 expression is not significantly different between Normal-like and Basal-like breast tumours (ANOVA;  $p>0.05$ ).

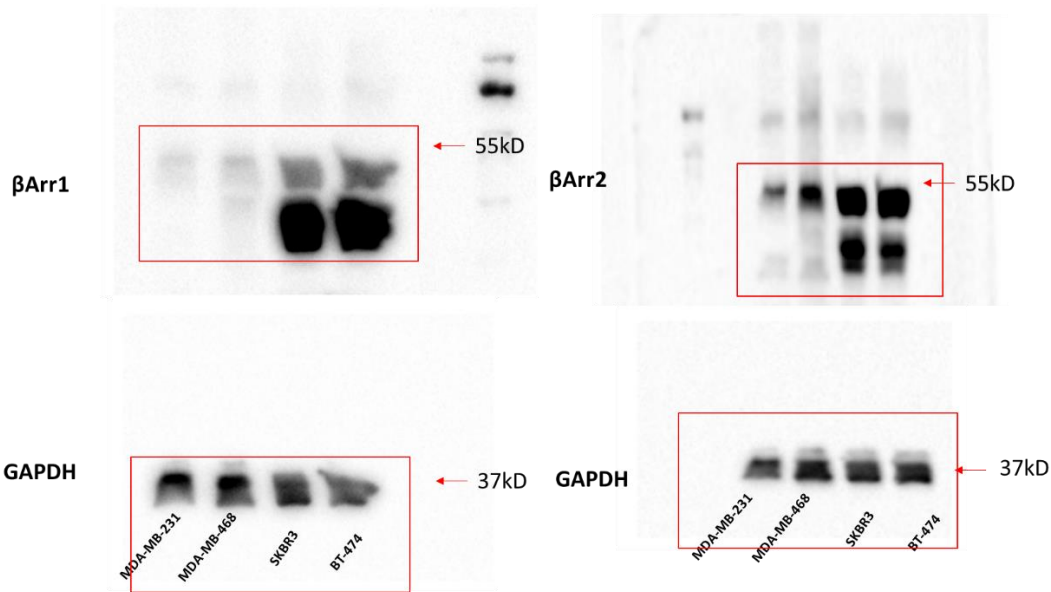


**Supplementary Figure 2. Silencing expression of  $\beta$ Arr1 or  $\beta$ Arr2 and cell invasion.**

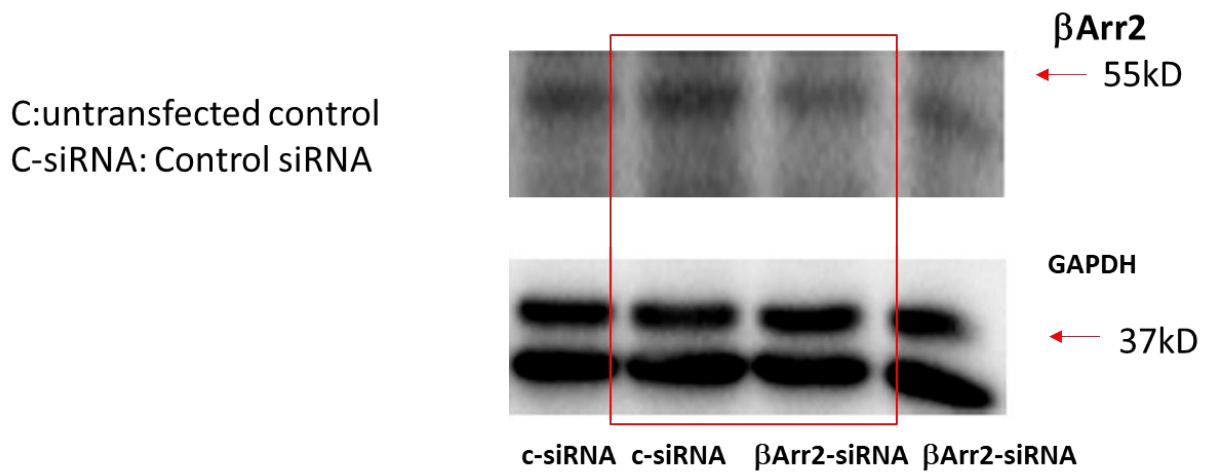
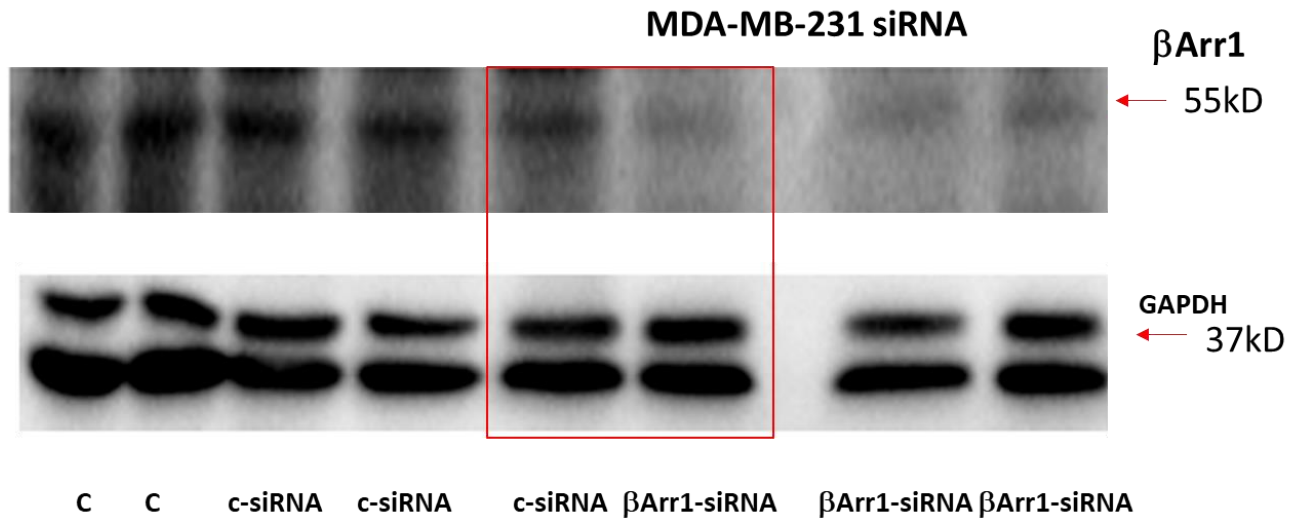
Invasion was significantly increased in  $\beta$ Arr1- or  $\beta$ Arr2-siRNA transfected MDA-MB-231 and MDA-MB-468 cells. Invasion of C (control-siRNA)-,  $\beta$ Arr1- or  $\beta$ Arr2-siRNA transfected cells was monitored continuously using an xCelligence Real-Time Cell Analyzer in Matrigel coated CIM-plates. The cells were seeded in the upper chamber of the plates and impedance measurements of cell invasion were recorded at 40 h. Y axis shows changes in impedance as a measurement of extent of cell invasion. Data are presented as mean  $\pm$  standard error of the mean; n=4-5; \* = P<0.05 versus C-siRNA.



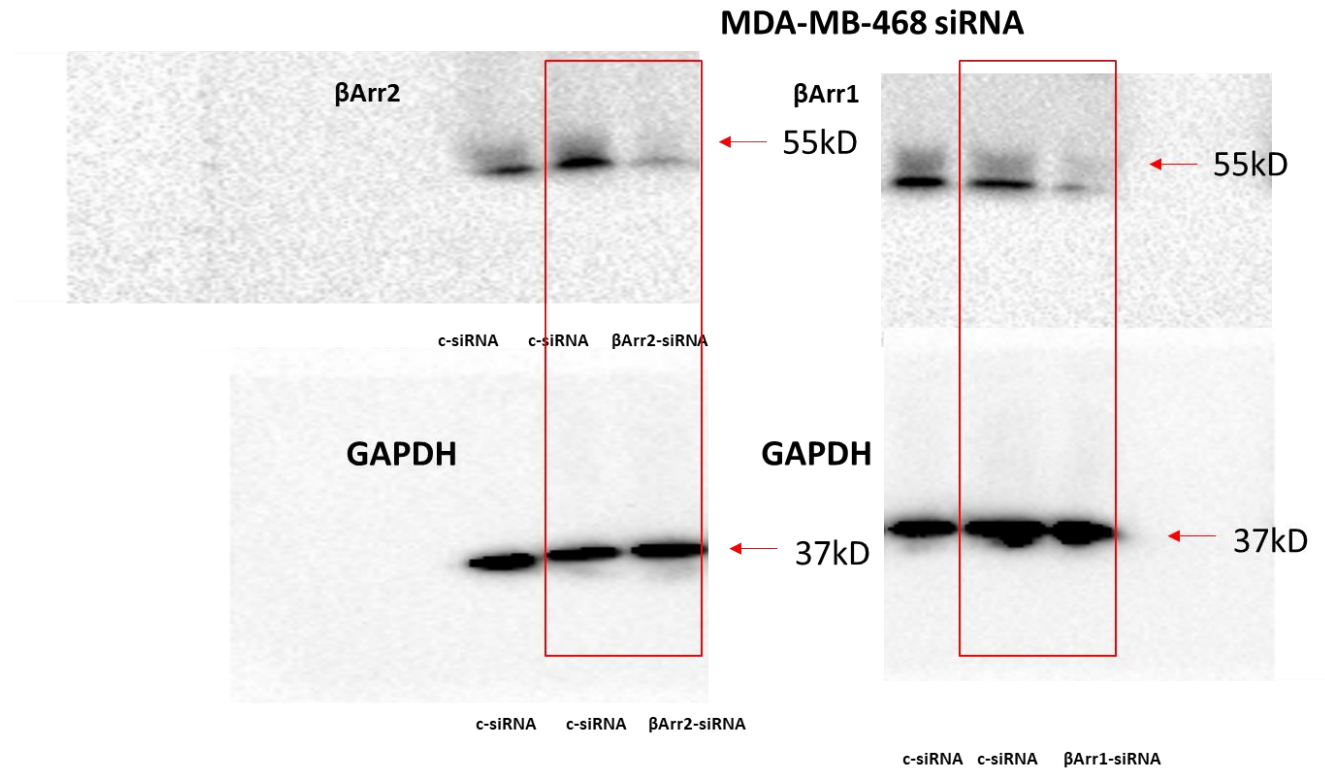
**Supplementary Figure 3.** In MDA-MB-231 and MDA-MB-468, transfection of  $\beta$ Arr1 or  $\beta$ Arr2-cDNA significantly decreased IGF-1R and Snail expression but did not change Cadherin and Vimentin expression. Bar graphs show the densitometric analysis of band intensities, normalized to GAPDH. Data are presented as mean  $\pm$  standard error of the mean; n=4-5; \* = P<0.05 versus C-cDNA.



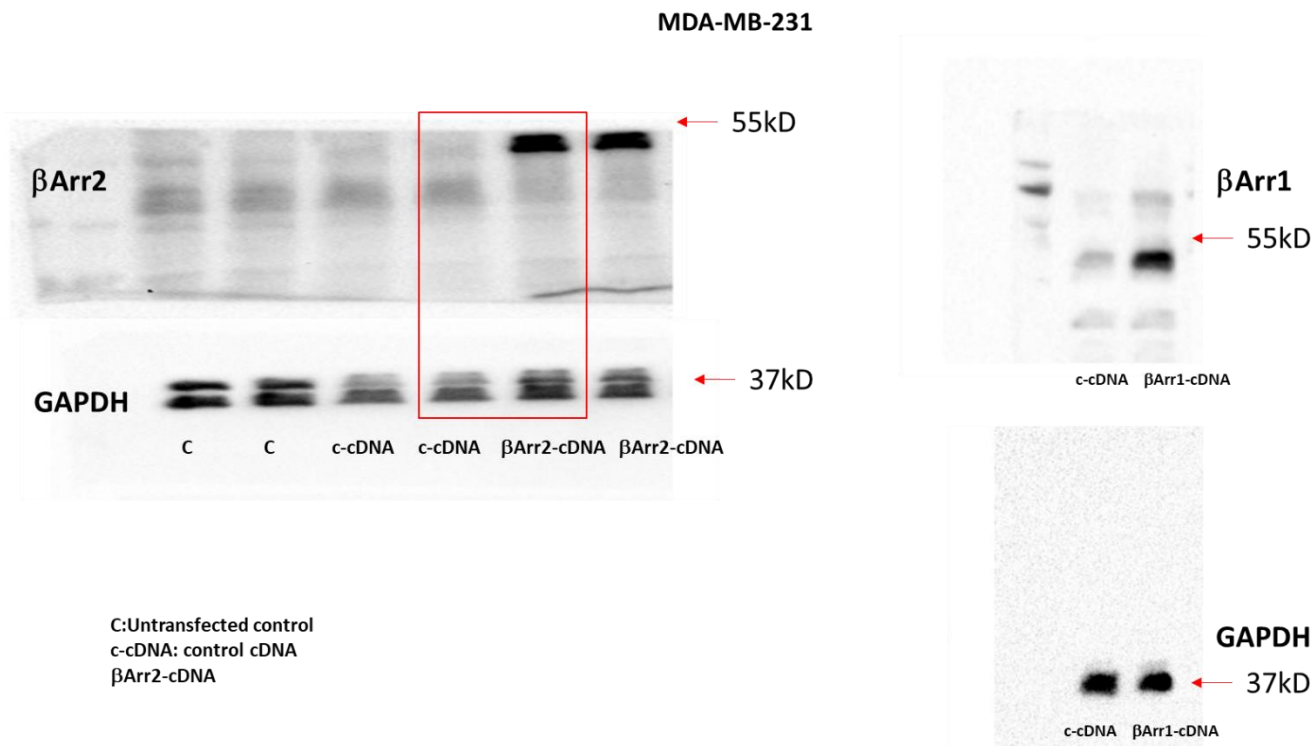
**Supplementary Figure 4.** The expression of  $\beta$ Arr1 or  $\beta$ Arr2 in the most aggressive breast cancer cell lines, TNBC cells (MDA-MB-231 and MDA-MB-468) and the less aggressive ones Her2 Enriched (SKBR3) and Luminal B (BT474). This supplementary figure is related to Figure 2.



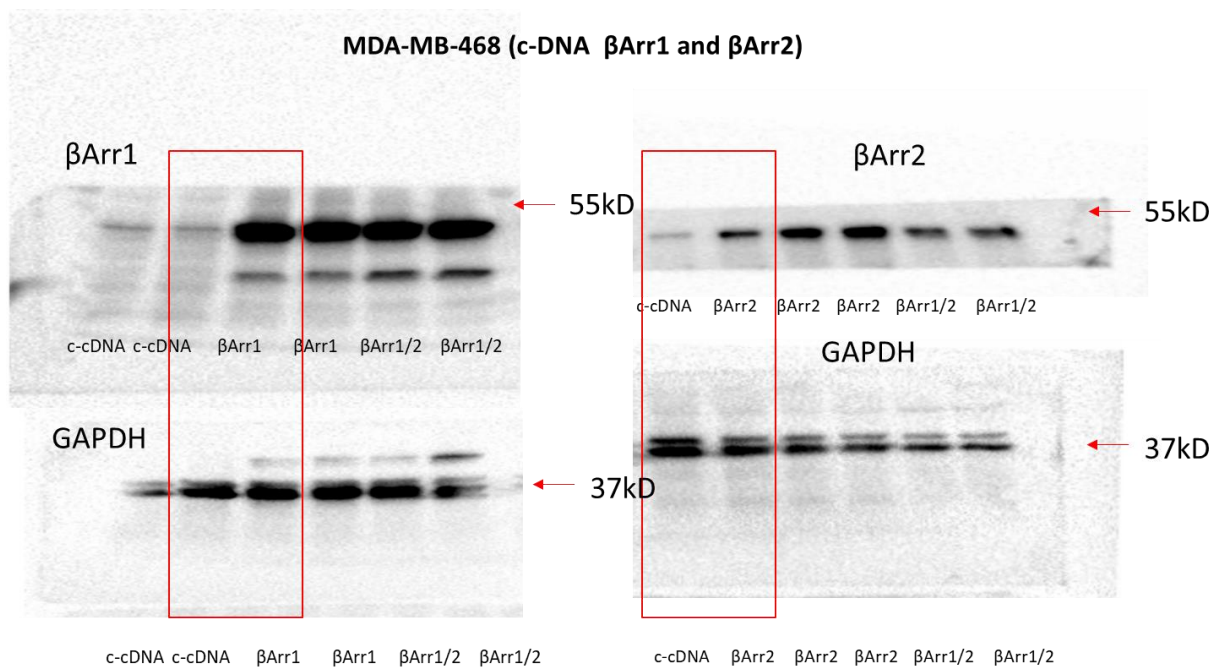
**Supplementary Figure 5.** The expression of βArr1 or βArr2 in control, control-siRNA, βArr1-siRNA or βArr2-siRNA transfected MDA-MB-231 cells. This supplementary figure is related to Figure 3.



**Supplementary Figure 6.** The expression of  $\beta$ Arr1 or  $\beta$ Arr2 in control, control-siRNA,  $\beta$ Arr1-siRNA or  $\beta$ Arr2-siRNA transfected MDA-MB-468 cells. This supplementary figure is related to Figure 3.

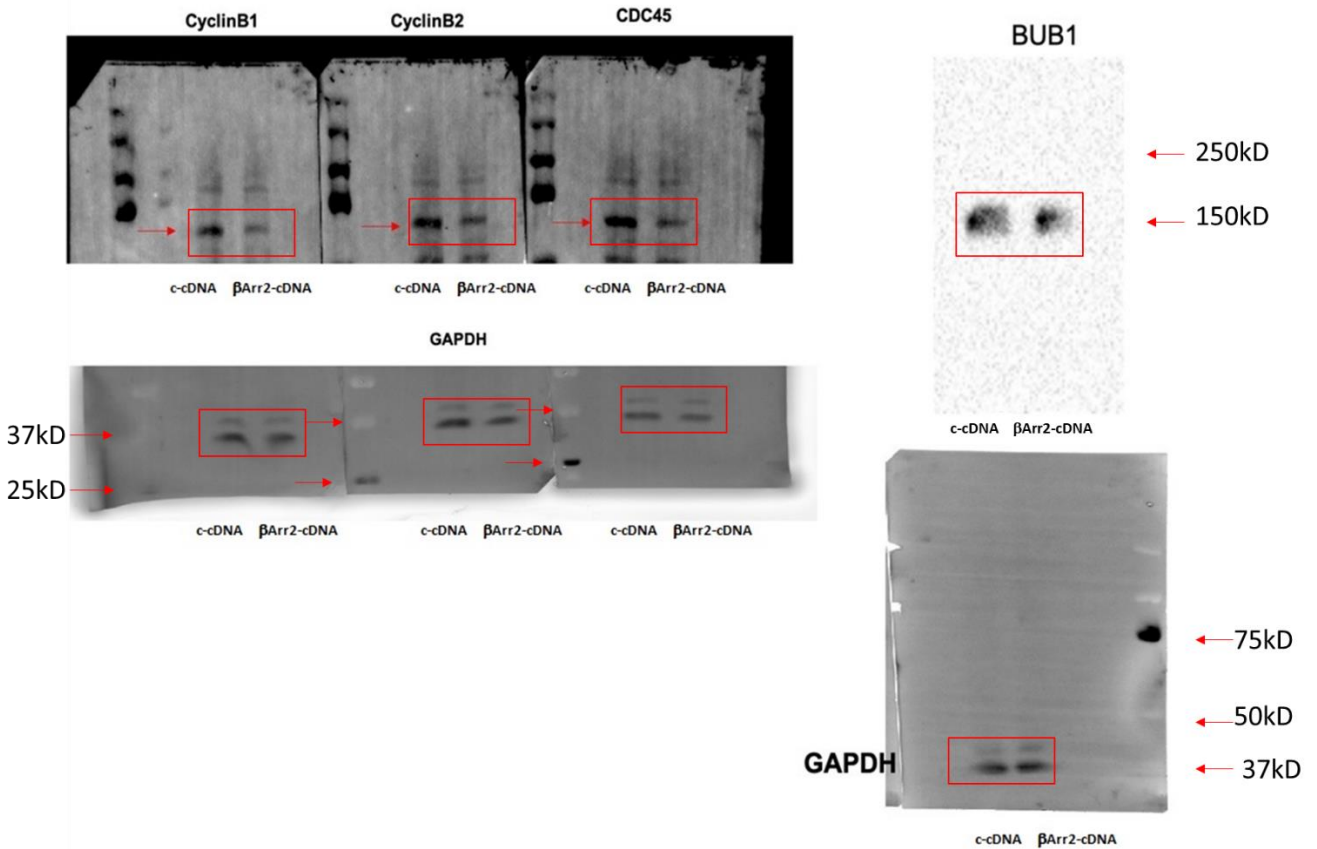


**Supplementary Figure 7.** The expression of βArr1 or βArr2 in control, control-cDNA, βArr1-cDNA or βArr2-cDNA transfected MDA-MB-231 cells. This supplementary figure is related to Figure 4.

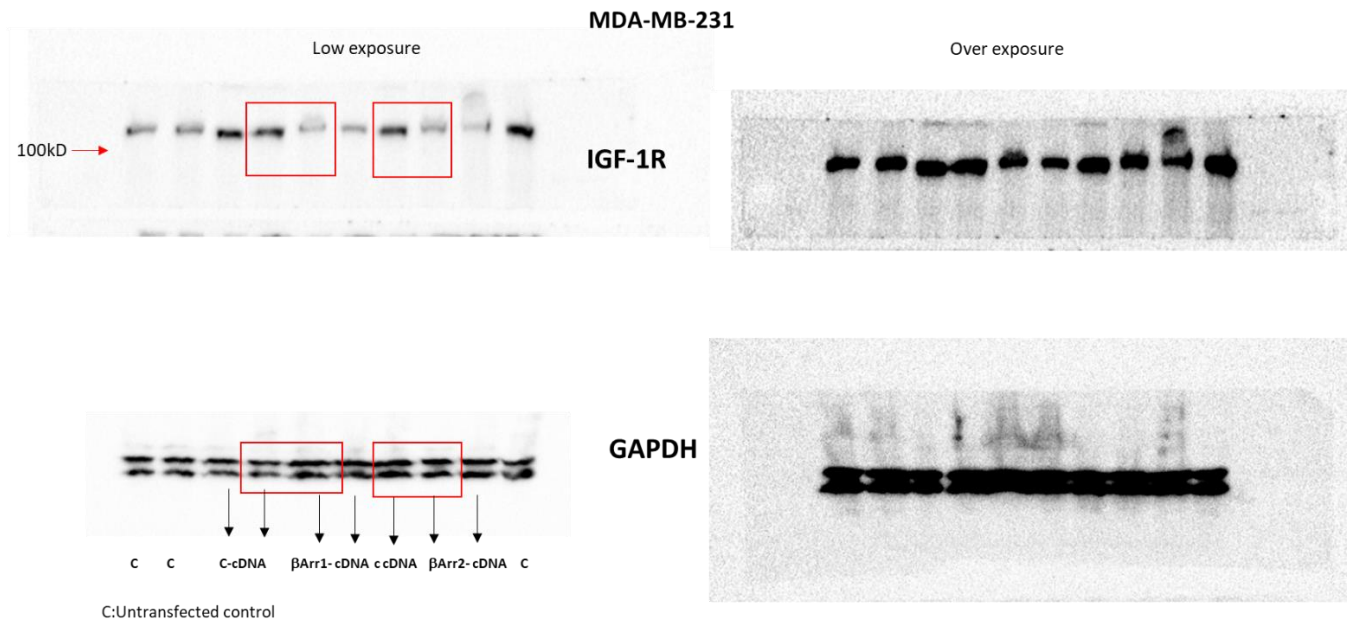




**Supplementary Figure 8.** The expression of  $\beta$ Arr1 or  $\beta$ Arr2 in control, control-cDNA,  $\beta$ Arr1-cDNA or  $\beta$ Arr2-cDNA transfected MDA-MB-468 cells. This supplementary figure is related to Figure 4.

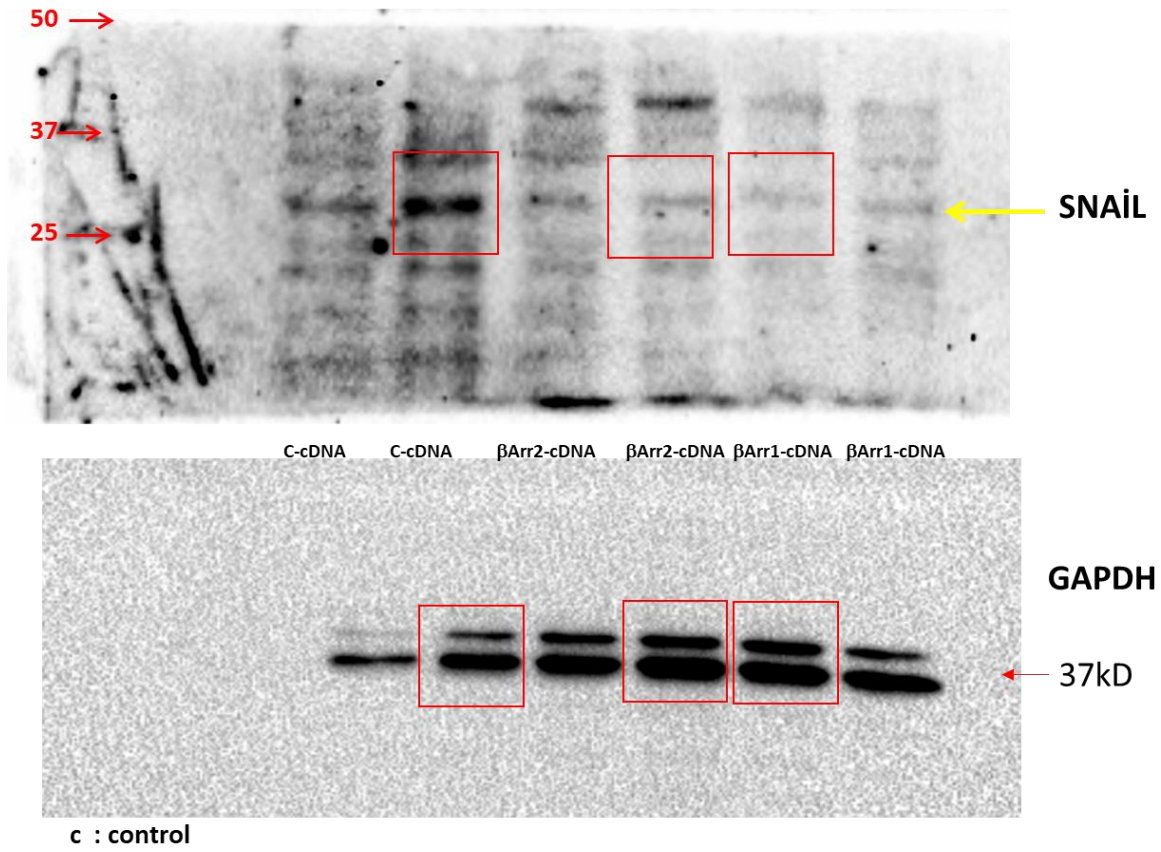


**Supplementary Figure 9.** The expression of CDC45, BUB1, CCNB1 and CCNB2 in control and  $\beta$ Arr1 overexpressed MDA-MB-231 cells. This supplementary figure is related to Figure 6.

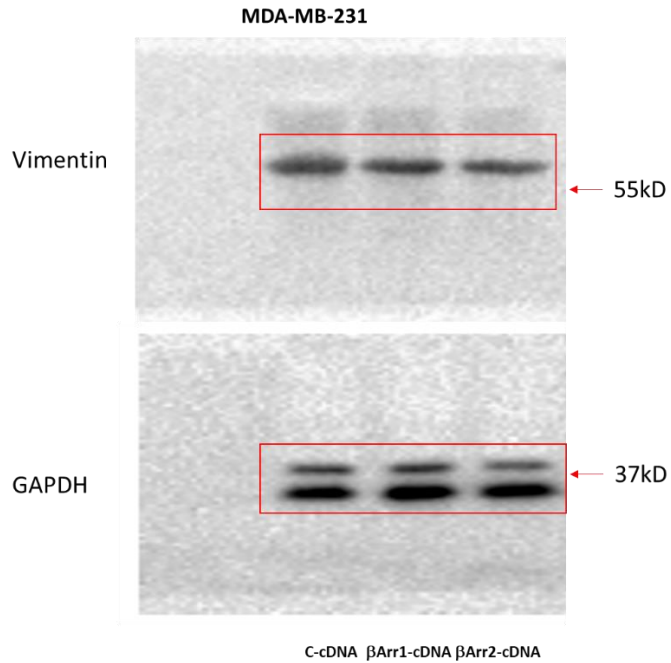


**Supplementary Figure 10.** The expression of IGF-1R in control (untransfected), control-cDNA,  $\beta$ Arr1-cDNA or  $\beta$ Arr2-cDNA transfected MDA-MB-231 cells. This supplementary figure is related to Figure 8.

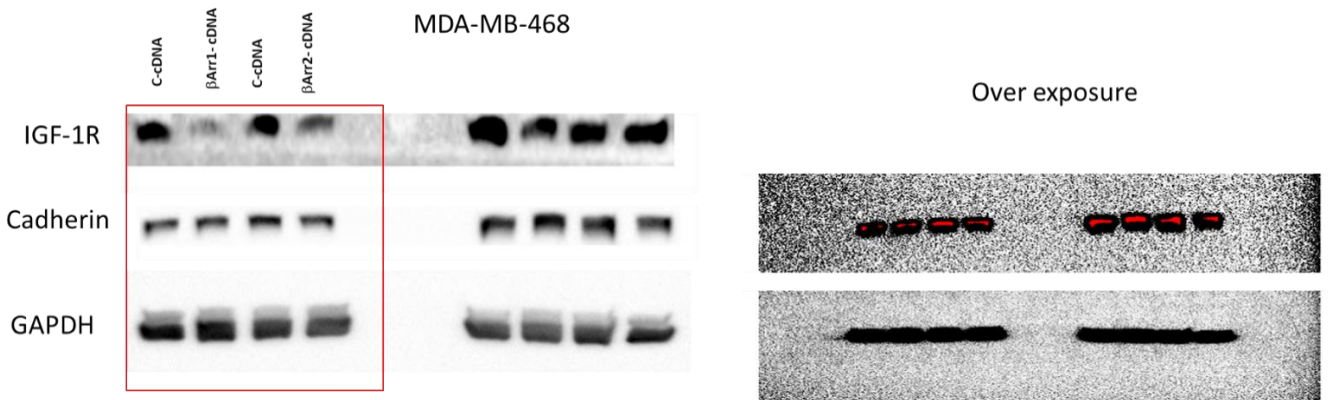
### MDA-MB-231



**Supplementary Figure 11.** The expression of Snail in control-cDNA,  $\beta$ Arr1-cDNA or  $\beta$ Arr2-cDNA transfected MDA-MB-231 cells. This supplementary figure is related to Figure 8.

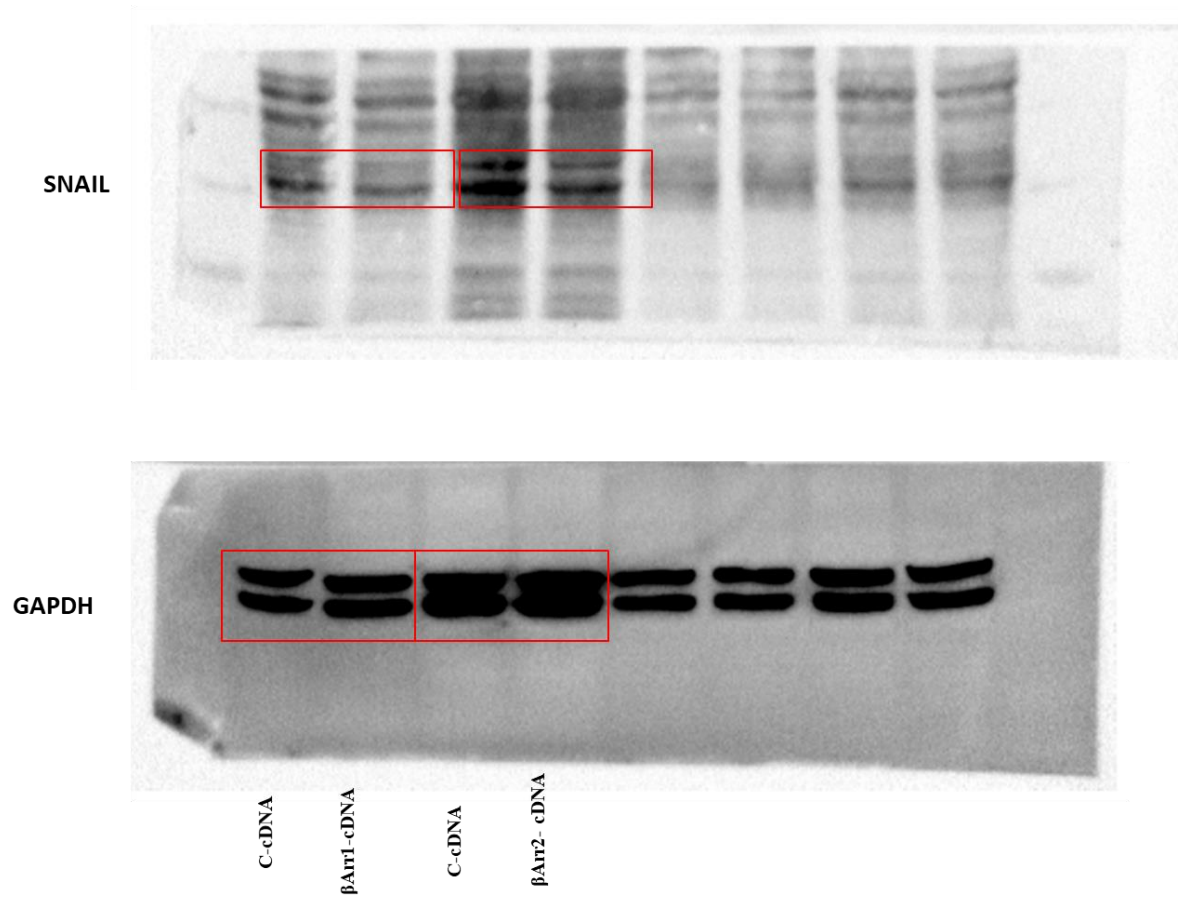


**Supplementary Figure 12.** The expression of vimentin in control-cDNA,  $\beta$ Arr1-cDNA or  $\beta$ Arr2-cDNA transfected MDA-MB-231 cells. This supplementary figure is related to Figure 8.



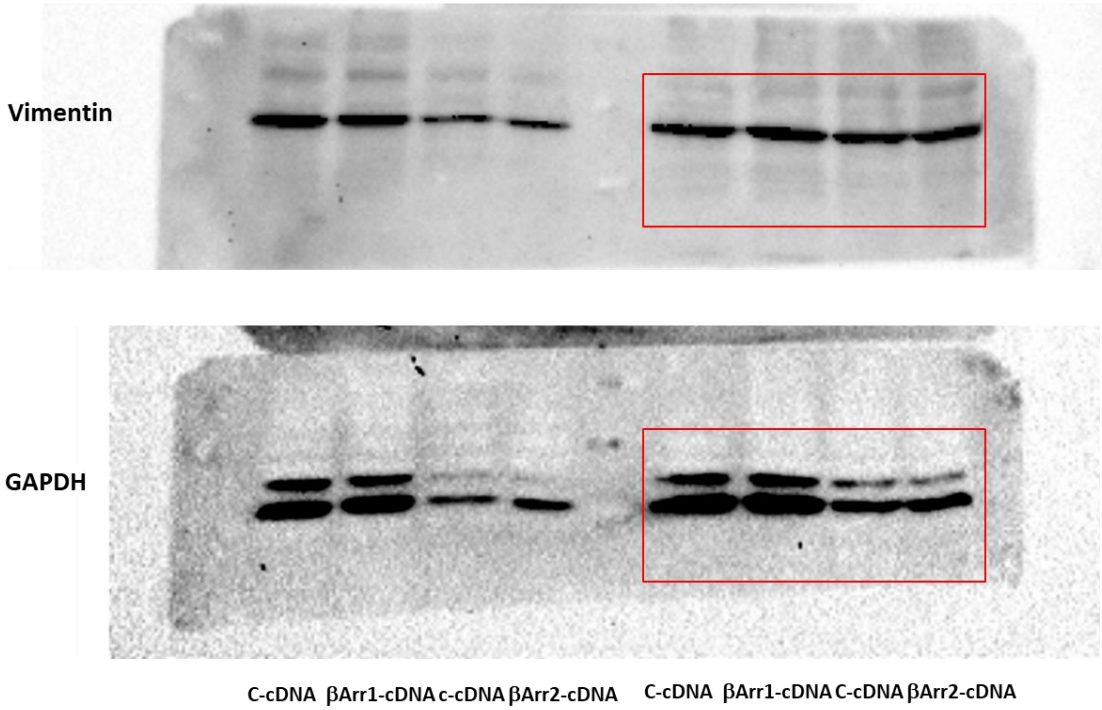
**Supplementary Figure 13.** The expression of IGF-1R and cadherin in control-cDNA,  $\beta$ Arr1-cDNA or  $\beta$ Arr2-cDNA transfected MDA-MB-468 cells. This supplementary figure is related to Figure 9.

MDA-MB-468



**Supplementary Figure 14.** The expression of snail in control-cDNA,  $\beta$ Arr1-cDNA or  $\beta$ Arr2-cDNA transfected MDA-MB-468 cells. This supplementary figure is related to Figure 9.

MDA-MB-468



**Supplementary Figure 15.** The expression of vimentin in control-cDNA,  $\beta$ Arr1-cDNA or  $\beta$ Arr2-cDNA transfected MDA-MB-468 cells. This supplementary figure is related to Figure 9.