

GABA promotes gastrin-releasing peptide secretion in NE/NE-like cells: Contribution to prostate cancer progression

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Supplementary Figure 1. Full-length blots/gels

Figure 1b

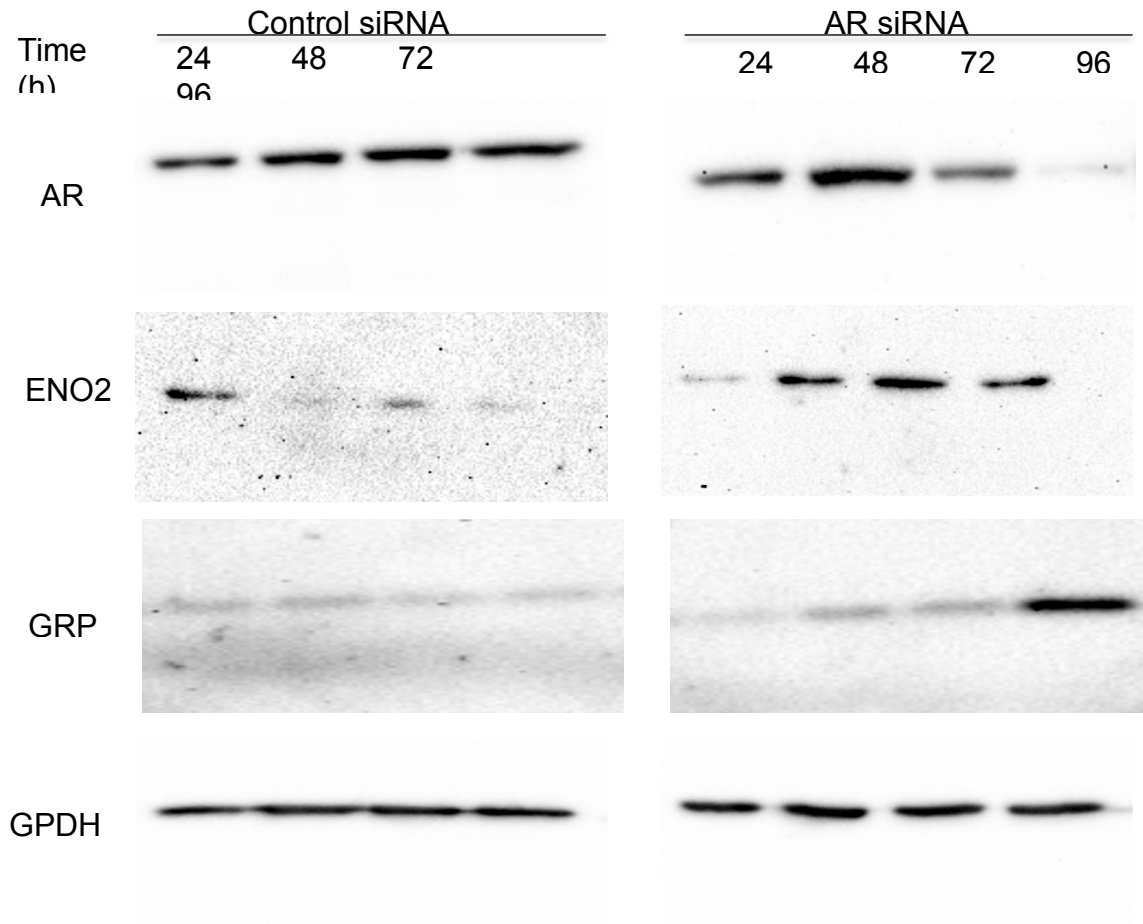


Figure 2d

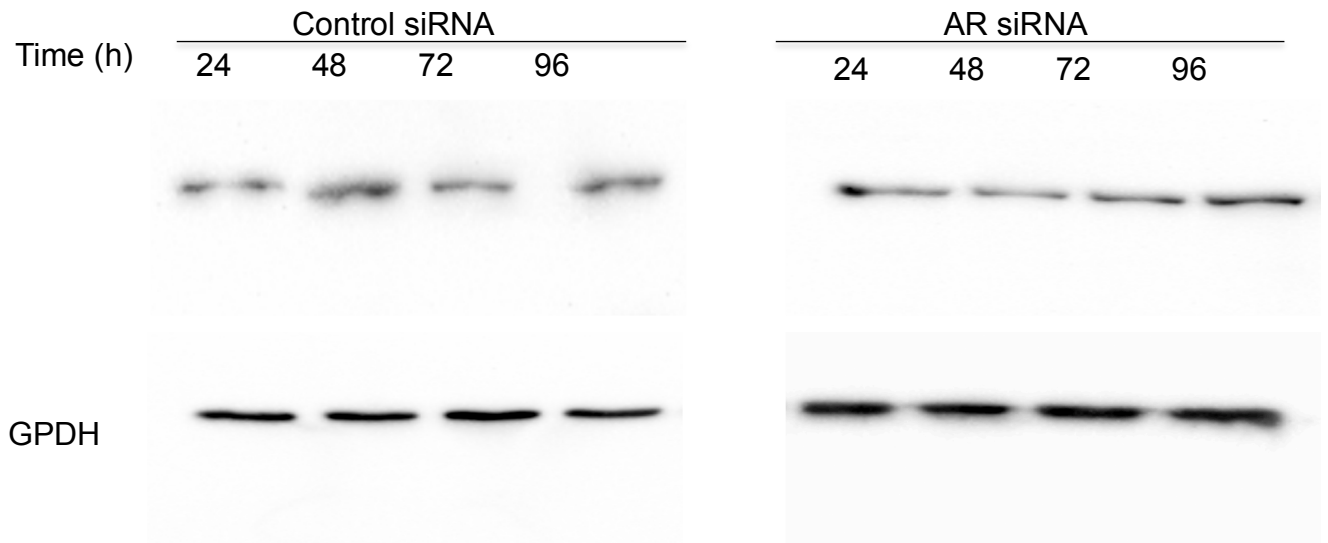
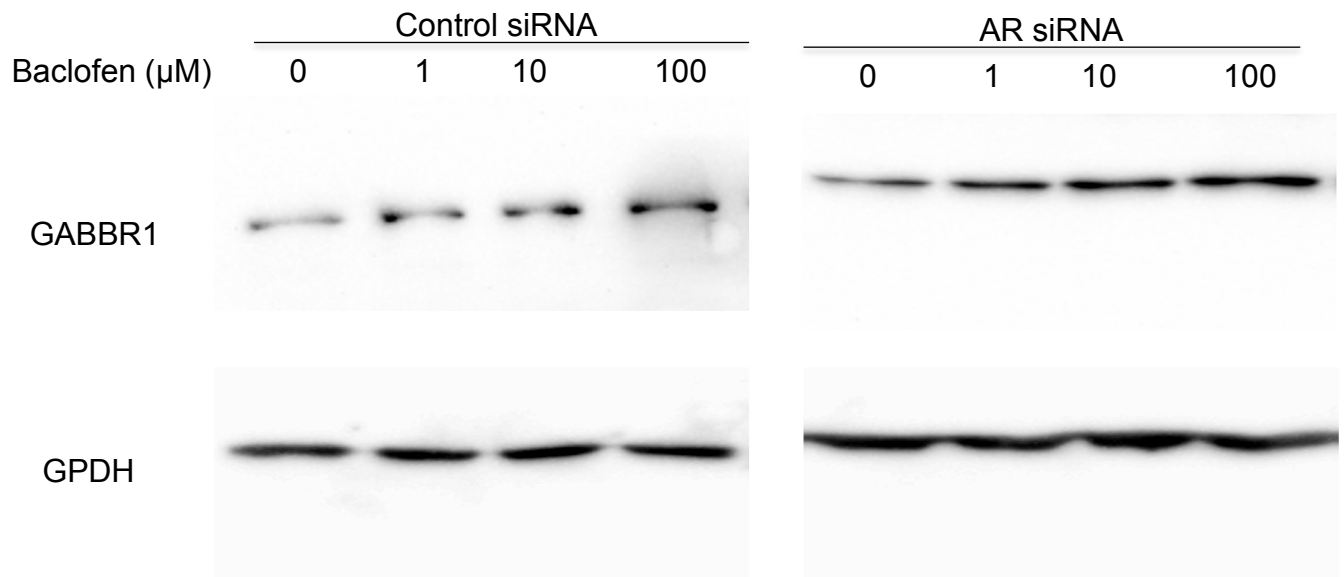
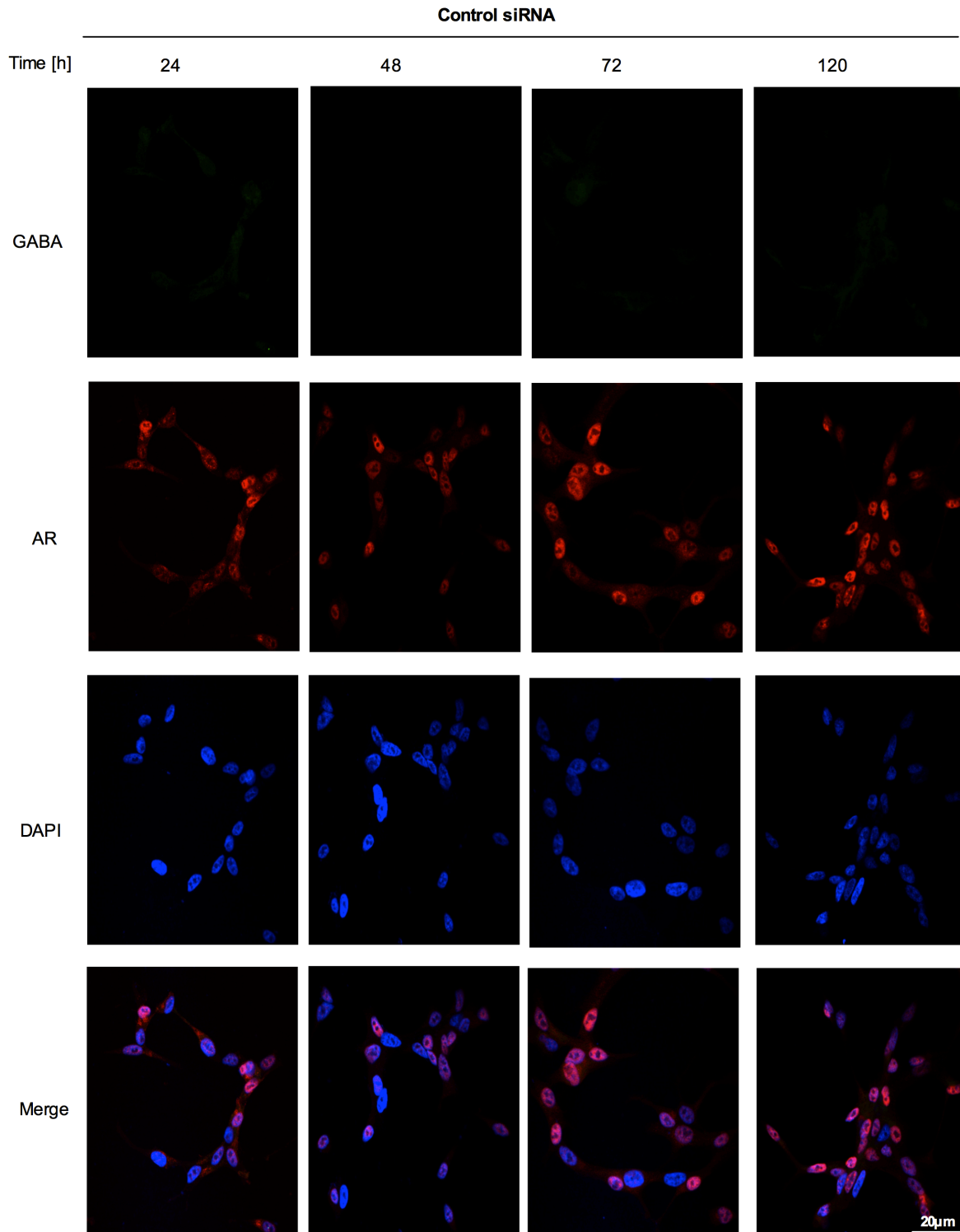


Figure 4b

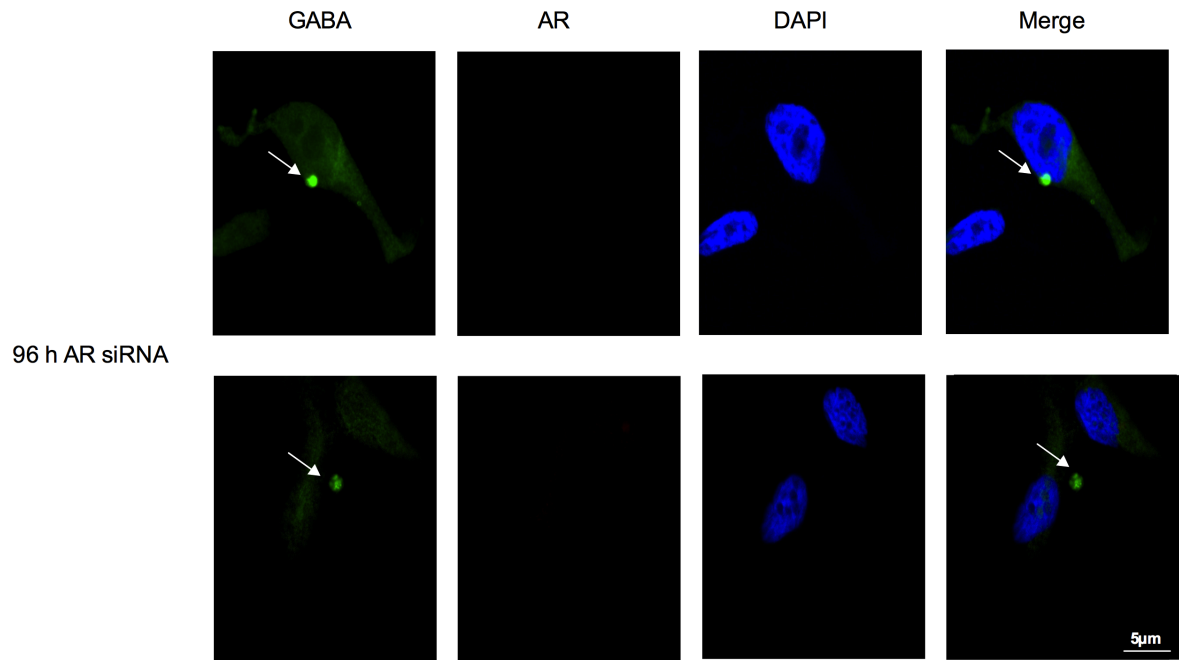


Supplementary Figure 2.



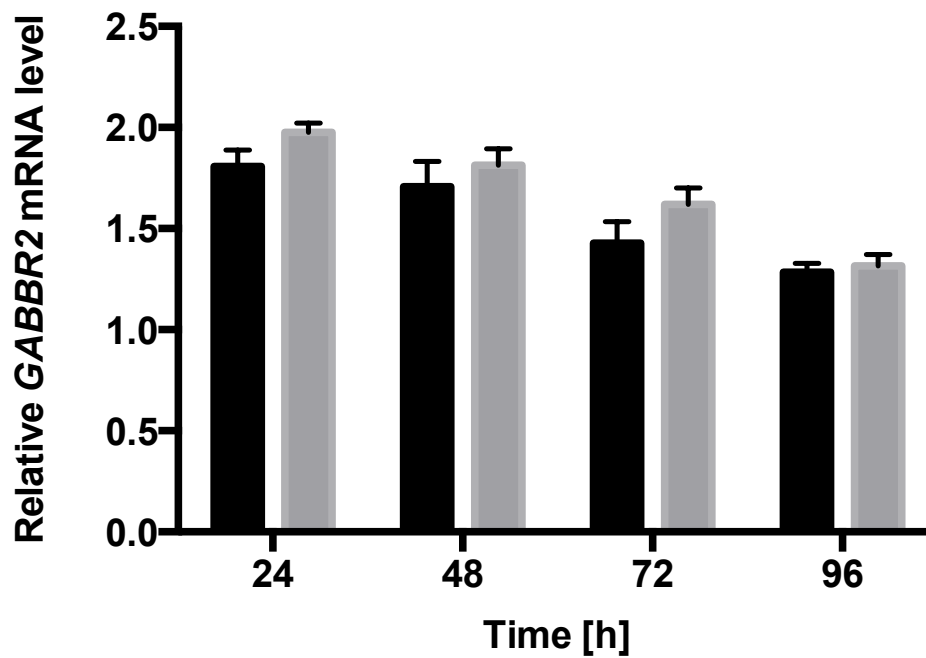
Supplementary Figure 2. Control siRNA. GABA levels are almost undetectable and AR content remains constant in control siRNA cells without morphological changes.

Supplementary Figure 3.



Supplementary Figure 3. Zoom of Figure 3a (scale_5µm).

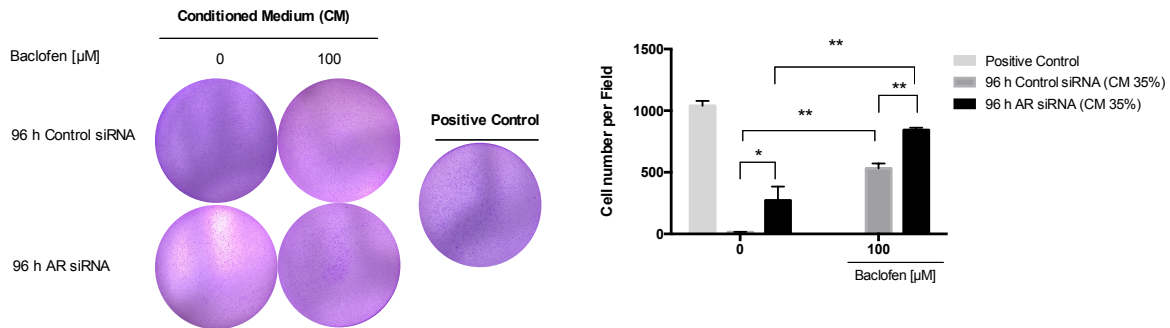
Supplementary Figure 4.



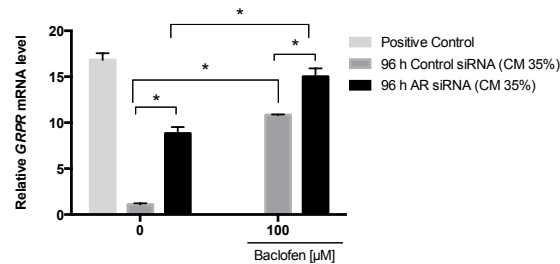
Supplementary Figure 4. GABBR2 mRNA levels. GABBR2 mRNA expression levels were analyzed by RT-qPCR in LNCaP cells transfected with AR-specific siRNA and control siRNA from 24 h to 96 h in RPMI supplemented with 10%FBS. Experiments were repeated three times. Differences among groups were compared by two-tailed unpaired Student's test. No significant differences were observed.

Supplementary Figure 5.

(a)



(b)



Supplementary Figure 5. The conditioned medium affects the invasive potential of NE-like cells through GRPR. a) Invasion assay was performed by using a Boyden chamber. AR knockdown LNCaP cells at 96 h (6×10^4) suspended in $200 \mu\text{l}$ of culture medium without FBS were placed in the upper chamber. $700 \mu\text{l}$ of conditioned medium from 96 h Control siRNA and 96 h AR siRNA treated with or without baclofen ($100 \mu\text{M}$) was added in the lower chamber for 24 h. Results are represented as mean \pm SEM of three independent experiments in triplicate wells * $p < 0.001$ and ** $p < 0.0001$. b) *GRPR* mRNA expression was analyzed by RT-qPCR. Results are presented as mean \pm SEM of three independent experiments in triplicate, * $p < 0.0001$. GRP synthesized was used as positive control; the previous concentration represents 35% of conditioned medium from 96 h AR siRNA treated with baclofen ($100 \mu\text{M}$) that was used to perform the experiments.