Phosphorylation of HSP90 by protein kinase A is essential for the nuclear translocation of androgen receptor

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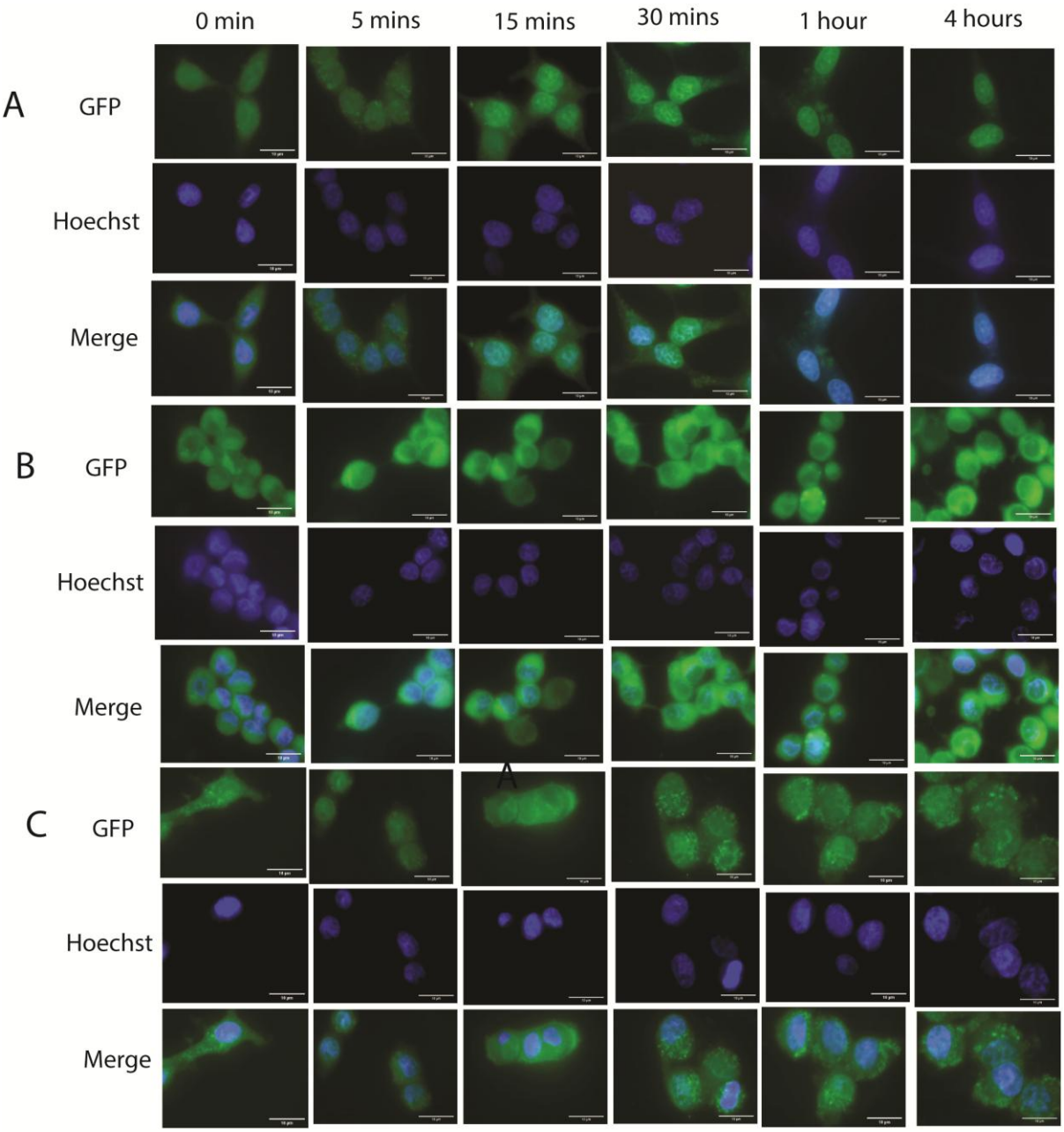

Figure S1

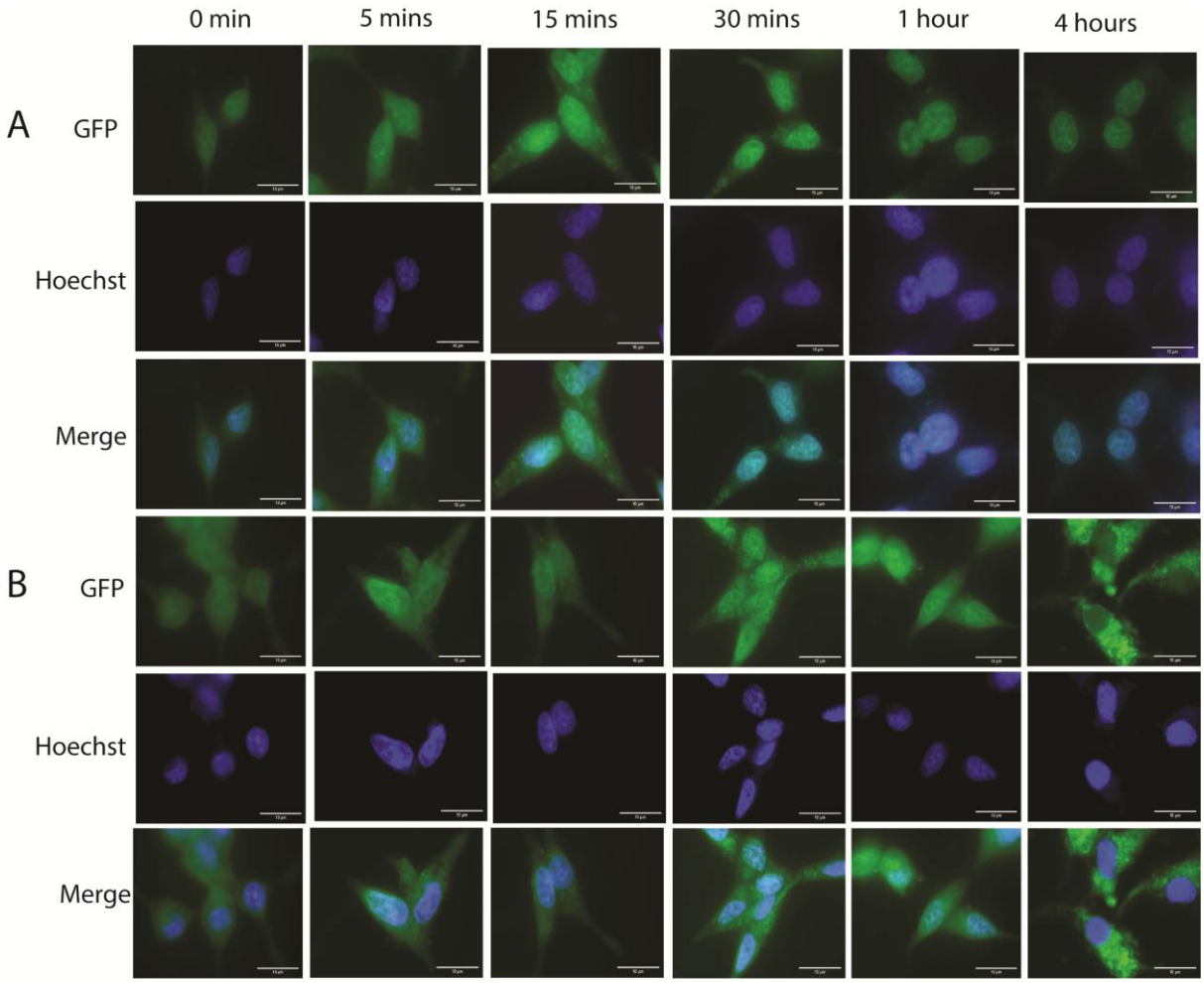

Figure S2

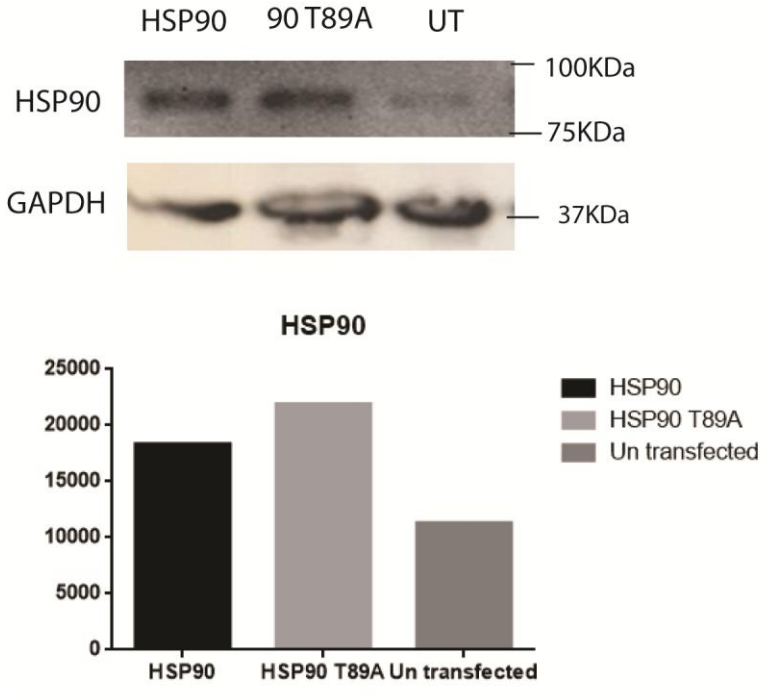

Figure S3

Figure Legends

Figure S1. Inhibition of AR translocation using PKA Inhibitor, H89 and by PKA-specific siRNA (SiPKA) in LNCaP cells. LNCaP cells were transfected using GFP-AR in presence or absence of siPKA (120nM). Transfected cells were treated or not treated with H89 (30uM) for 40 mins prior to treatement with testosterone (10nM). **A**. Images at different time intervals (0, 5, 15, 30mins, 1 and 4 hours) after treatment with Testosterone. **B.** Images after treatment with testosterone in the presence of H89. **C**. Images of siPKA transfected cells after treatment with Testosterone. Scale bar, 10µm (added using Image J). Results for two of these time points, namely, 0 and 60 mins have been highlighted in Figure 2, to demonstrate that migration of AR into the nucleus is almost complete by 60 mins post- testosterone treatment, and is inhibited if PKA activation is inhibited by H89 or siPKA.

Figure S2. Inhibition of AR translocation in HSP90 T89A transfected LNCaP cells. LNCaP cells were transfected using GFP-AR alone or in combination with HSP90 T89A mutant. Transfected cells were treated with testosterone (10nM) and images were captured. **A.** Images at different time intervals (0, 5, 15, 30mins, 1 and 4 hours) after treatment with Testosterone (10nM). **B**. Images after treatment with Testosterone in HSP90 T89A transfected cells. Scale bar, 10µm (added using Image J). Results for two of these time points, namely, 0 and 60 mins have been highlighted in Figure 5, to demonstrate that migration of AR in to the nucleus is almost complete by 60 mins post-testosterone treatment and is inhibited in HSP90 T89A expressing cells.

Figure S3. A. Anti-HSP90 antibody detects both HSP90 and HSP90 T89A mutant proteins. LNCaP cells were transfected using HSP90/ HSP90 T89A or vector alone . Western blot was performed using HSP90 antibody. GAPDH was used as loading control.