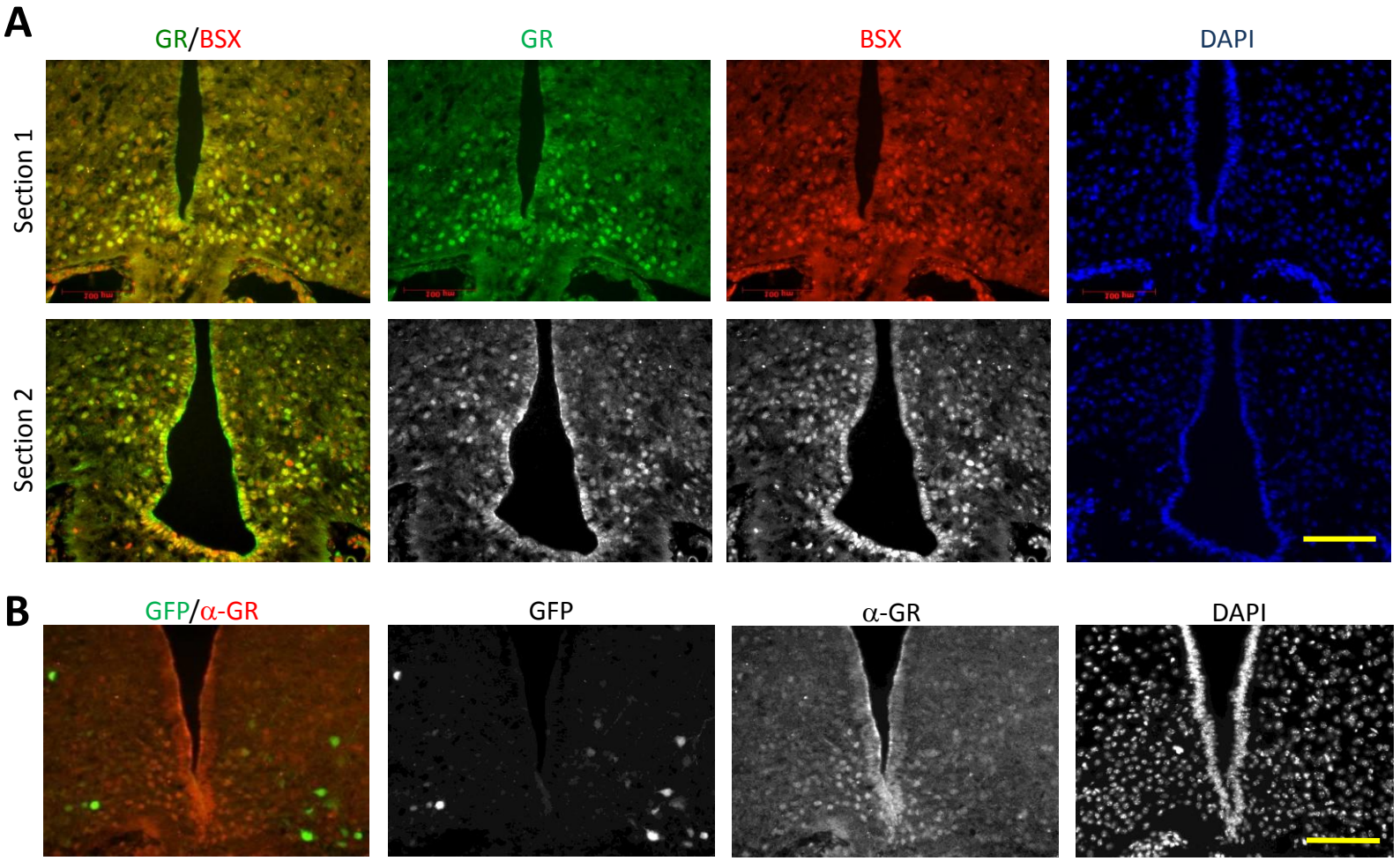

Figure S1 GR Expression in AgRP-neurons but not in POMC-neurons. (A) The color images shown in Fig. 1A are shown here in section 1. The images in section 2 are for an ARC location more rostral to the section 1. Although images similar to the section 2 images are typically used as representative AgRP-neuron-enriched images, more cuadal locations (like the above section 1 images) contain even more AgRP-neurons, as apparent in these images. (B) The ARC sections of GFP-POMC transgenic mice were immunostained with anti-GR antibody. GFP^+ POMC neurons did not express GR. POMC- and AgRP-neurons are known to be located in the lateral part of the ARC and near the 3rd ventricle, respectively. In support of the fact that GFP^+ cells are indeed POMC-neurons and GR^+ cells are AgRP-neurons, GFP^+ and GR^+ cells are located in the lateral part of the ARC and near the 3rd ventricle, respectively. The yellow scale bar in the DAPI panel is for 100 μm.

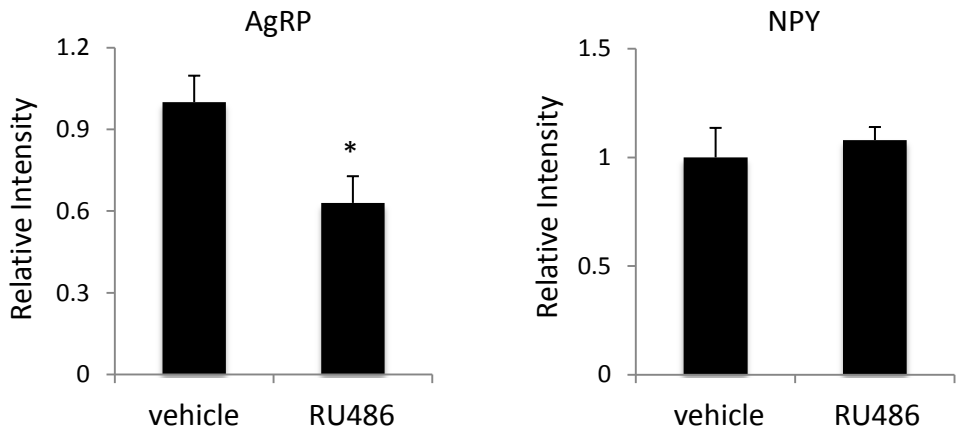

Figure S2 Intracerebroventricular (ICV) injection of GR antagonist, RU486. RU486 was introduced to the ARC via ICV injection (n=3 for each group), followed by in situ hybridization (ISH) for AgRP an NPY. The ISH signal intensity of serial coronal 12 μm sections of the ARC region was measured using ImageJ and plotted against the intensity of vehicle control samples.

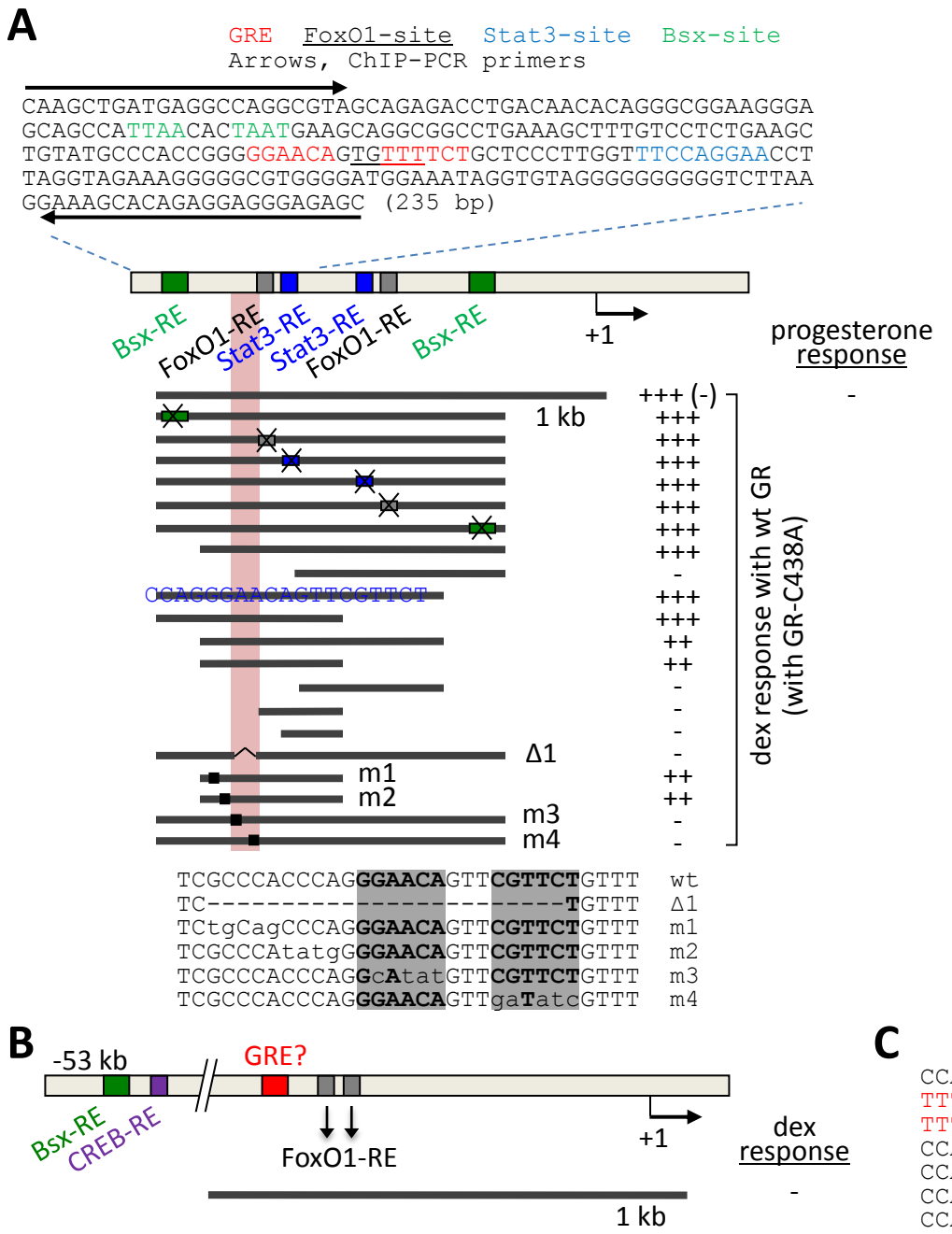

Figure S3 Analysis of *AgRP/NPY* Promoters. (A) Deletions and point-mutations were introduced to human *AgRP* promoter, which enabled us to map the AgRP-GRE (sequences shaded in gray). Both the Dex response and the synergy with Bsx were inseparable and required the AgRP-GRE. Dex failed to activate *AgRP* 1kb promoter in the presence of GR-C438A, a mutant GR incapable of binding to GRE. Progesterone also failed to activate *AgRP* 1kb promoter. Two primers for mouse hypothalamic ChIP assays are as shown, which produce a PCR product of 235 bp containing not only the AgRP-GRE but also the upstream Bsx-, FoxO1- and Stat3-sites. (B) Schematics for NPY promoter and the known response elements. The 1 kb NPY promoter containing a putative GRE did not respond to Dex. (C) Hybrid AgRP-GRE motifs to map critical nucleotides in AgRP-GRE (see text).

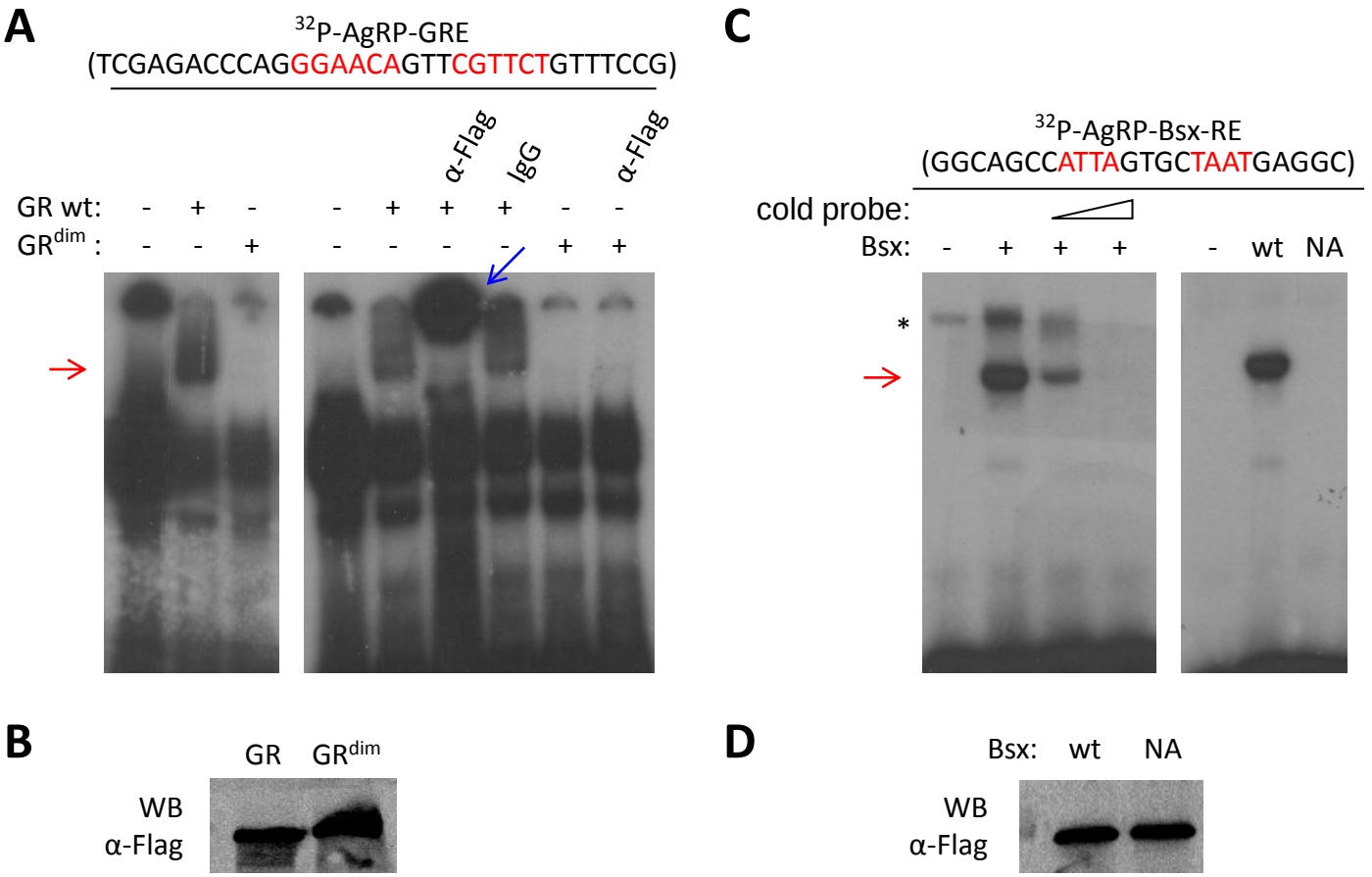

Figure S4 Binding of *AgRP* promoter by GR and Bsx. Gel shift assays were carried out to show binding of GR and Bsx to AgRP-GRE (A) and Bsx-response elements (C), respectively. * indicates a non-specific binding. Red and blue arrows indicate specific GR or Bsx band (red) and super-shifted GR band (blue), respectively. GR^{dim} indicates a dimerization-defective (hence a DNA-binding defective) mutant GR, GR-A458T. NA indicates N160A, a DNA-binding defective mutant Bsx protein with an 'N to A' mutation at the 160^{th} amino acid in the DNA binding domain. The sequences of the probes are as shown (response elements highlighted in red). (B, D) Western blotting of in vitro translated Flag-tagged GR and Bsx proteins used in the gel shift assays.

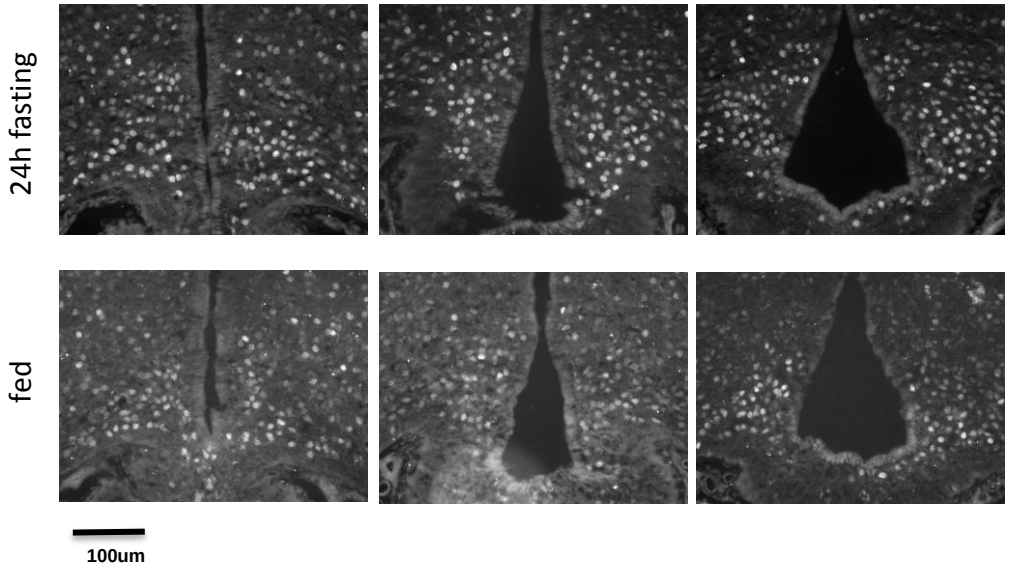

Figure S5 Immunostining of Bsx in the ARC. The coronal sections of the ARC region of wild-type mice, either fed (lower row) or fasted for 24h (upper row), were immunostained with antibody against Bsx. Bsx levels were enhanced upon fasting as reported (Nogueiras et al. Endocrinology 149:3009, 2008).

Ref.	GREs	+Dex	BSX+ DEX
1	mBcl-X_L (I)	+	-
	mBcl-X_L (II)	-	NA
	mBcl-X_L (III)	-	NA
2	TAT	+	-
3	Dax-1	+	-
4	MuRF1	+	-
5	ASBT (I)	+	-
	ASBT (II)	+	-
6	G6Pase (I, II)	-	NA
	G6Pase (III)	-	NA
7	β2-AR	+	-
8	TH	+	-
9	Sgk1	-	NA
10	ENaCα	-	NA
11	MT-1 (I)	+	-
	MT-1 (II)	+	-

Figure S6 Bsx as a Negative Regulator of GREs. 2-4 copies of the known GREs from the genes referenced below were cloned into the TK-LUC reporter. The response of these reporters to Dex alone or Dex+Bsx was examined in HEK293 cells. 10 out of 16 GREs directed robust Dex response (indicated as + in +Dex column) and all of these Dex responses were potently suppressed by Bsx (indicated as – in Bsx+Dex column). GREs that did not respond to Dex were not affected by the presence of Bsx (NA).

1. Gascoyne, D. M., Kypta, R. M., and Vivanco, M. M. (2003). Glucocorticoids inhibit apoptosis during fibrosarcoma development by transcriptionally activating Bcl-x_L. J. Biol. Chem. *278*, 18022–29.
2. Jantzen, H. M., Strähle, U., Gloss, B., Stewart, F., Schmid, W., Boshart, M., Miksicek, R., and Schütz, G. (1987). Cooperativity of glucocorticoid response elements located far upstream of the tyrosine aminotransferase gene. Cell *49*, 29-38.
3. Gummow, B. M., Scheys, J. O., Cancelli, V. R., and Hammer, G. D. (2006). Reciprocal regulation of a glucocorticoid receptor-steroidogenic factor-1 transcription complex on the *Dax-1* promoter by glucocorticoids and adrenocorticotropic hormone in the adrenal cortex. Mol. Endocrinol. *20*, 2711-23.
4. Waddell, D. S., Baehr, L. M., van den Brandt, J., Johnsen, S. A., Reichardt, H. M., Furlow, J. D., and Bodin, S. C. (2008). The glucocorticoid receptor and FOXO1 synergistically activate the skeletal muscle atrophy-associated MuRF1 gene. Am. J. Physiol. Endocrinol. Metab. *295*, E785–97.
5. Jung, D. Fantin, A. C., Scheurer, U., Fried, M., and Kullak-Ublick, G. A. (2004). Human ileal bile acid transporter gene *ASBT* (*SLC10A2*) is transactivated by the glucocorticoid receptor. Gut *53*, 78–84.
6. Yoshiuchi, I., Shingu, R., Nakajima, H., Hamaguchi, T., Horikawa, Y., Yamasaki, T., Oue, T., Ono, A., Miyagawa, J. I., Namba, M., et al. (1998). Mutation/polymorphism scanning of glucose-6-phosphatase gene promoter in noninsulin-dependent diabetes mellitus patients. J. Clin. Endocrinol. Metab. *83*, 1016-9.
7. Cornett, L. E., Hiller, F. C., Jacobi, S. E., Cao, W., and McGraw, D. W. (1998). Identification of a glucocorticoid response element in the rat beta2-adrenergic receptor gene. Mol. Pharmacol. *54*, 1016-23.
8. Hagerty, T., Fernandez, E., Lynch, K., Wang, S. S., Morgan, W. W., and Strong, R. (2001). Interaction of a glucocorticoid-responsive element with regulatory sequences in the promoter region of the mouse tyrosine hydroxylase gene. J. Neurochem. *78*, 1379-88.
9. Webster, M. K., Goya, L., Ge, Y., Maiyar, A. C., and Firestone, G. L. (1993). Characterization of sgk, a novel member of the serine/threonine protein kinase gene family which is transcriptionally induced by glucocorticoids and serum. Mol. Cell. Biol. 13, 2031-40.
10. McTavish, N., Getty, J., Burchell, A., and Wilson, S. M. (2009). Glucocorticoids can activate the alpha-ENaC gene promoter independently of SGK1. Biochem. J. *423*, 189-97.
11. Ghoshal, K., Wang, Y., Sheridan, J. F., and Jacob, S. T. (1998). Metallothionein induction in response to restraint stress: Transcriptional control, adaptation to stress, and role of glucocorticoid. J. Biol. Chem. *273*, 27904–10. a

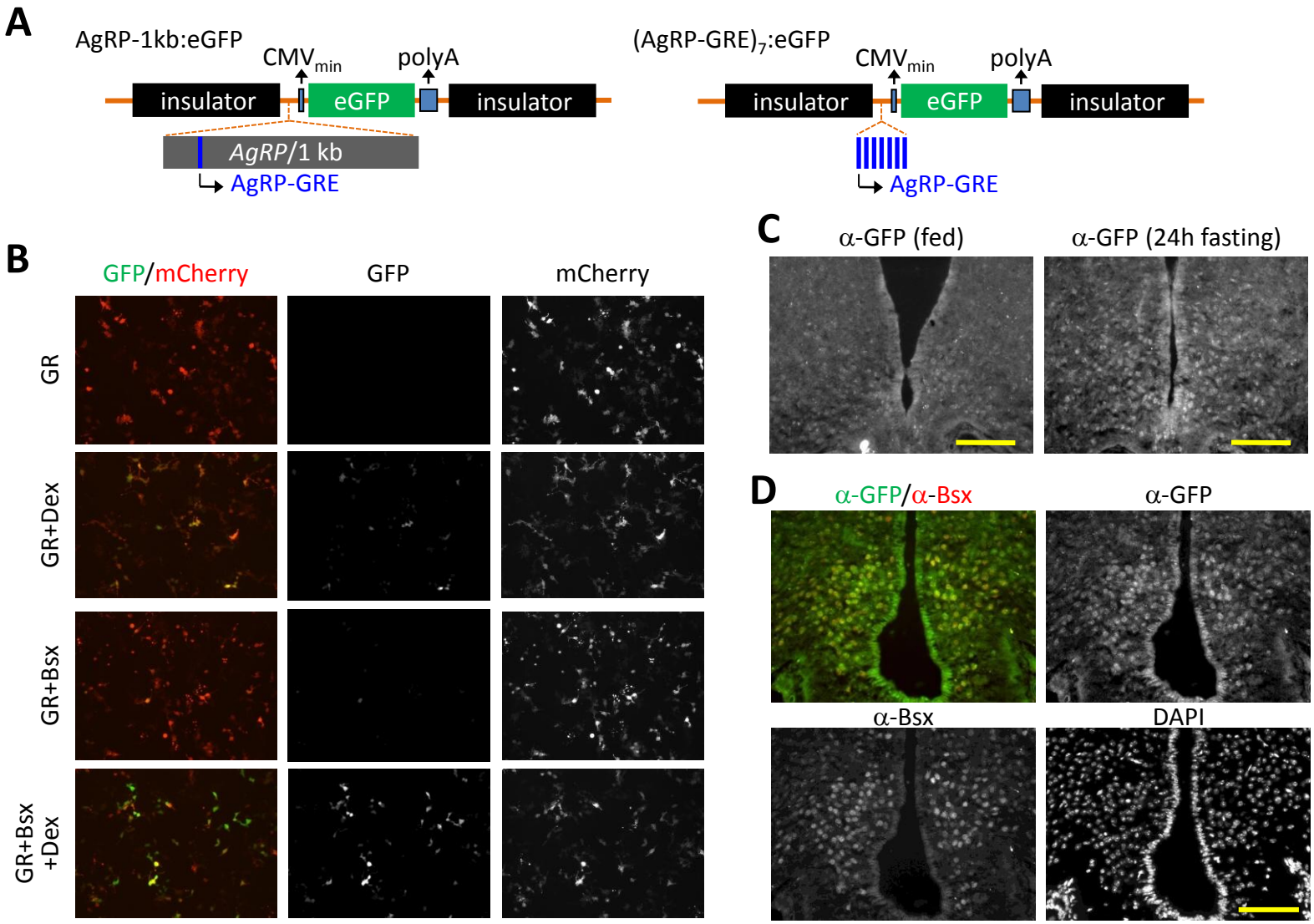

Figure S7 AgRP-GRE-directed Expression of GFP in HEK293 and AgRP-neurons. (A) Schematics of AgRP-1kb:eGFP and (AgRP-GRE)$_7$:eGFP reporters. (B) In HEK293 cells transfected with GR-expression vector, AgRP-1kb:eGFP reporter directed GFP expression in a Dex-dependent manner. Coexpression of Bsx significantly potentiated the Dex response. mCherry was used as a transfection indicator. Similar results were also obtained with (AgRP-GRE)$_7$:eGFP reporter. (C, D) The coronal sections of the ARC region of transgenic mice with (AgRP-GRE)$_7$:eGFP, either fed (C) or fasted for 24h (C, D), were immunostained with antibodies against GFP and Bsx, as indicated.

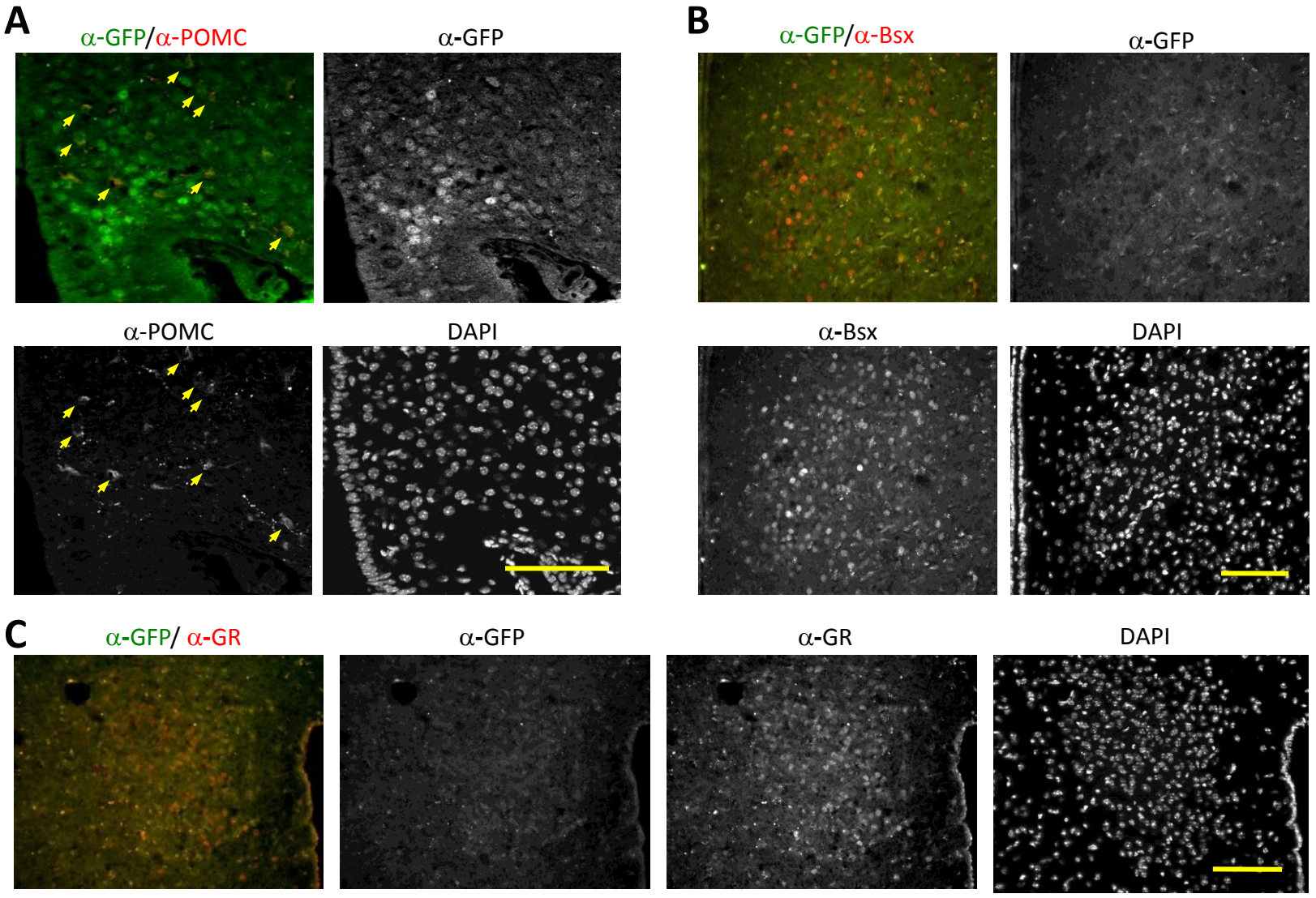

Figure S8 Lack of GFP Expression in POMC-neurons, the DMH, and the PVN. (A) GFP$^+$ cells in the ARC of AgRP-1kb:eGFP transgenic mice were not immunostained with anti-POMC antibody. Of note, POMC antibody immunostained not only POMC-neuronal cell bodies (indicated as yellow arrows) but also axons and synaptic termini. (B, C) GFP expression was hardly detected either in the DMH (B) or in the PVN (C), while the DMH and the PVN expressed Bsx and GR, respectively. The yellow scale bars denote 100 μm (A-C).