

## SUPPLEMENTAL FILE

**Title:** Dynamic *Ins2* gene activity defines  $\beta$  cell maturity states

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**Supplemental Table 1:** Primary antibodies

Antibody Name	Source Species	Company	RRID	Dilution IHC
Insulin	Guinea Pig	Abcam Cat# Ab7842	AB_306130	1/100
GFP	Rabbit	Thermo Fisher Cat# A11122	AB_221569	1/1000
PDX1	Rabbit	Abcam Cat# Ab47267	AB_777179	1/2000
GFP	Chicken	USbiological life sciences Cat#168231	AB_2307313	1/200
CHGA	Sheep	Biomol International Cat#CA1128	AB_2291864	1:200
CLOCK	Rabbit	Thermo Scientific Cat#MA5-32713	AB_1809990	1/50
GRP78	Rabbit	Abcam Cat# Ab21685	AB_2119834	1/500
Proinsulin	Human	R&D systems Cat#MAB13361	AB_2126534	1/200

**Supplemental Table 2:** qPCR primer sequences

	Forward	Reverse
<i>Ins1</i>	5'TAGTGACCAGCTATAATCAGAG3'	5'ACGCCAAGGTCTGAAGGTCC3'
<i>Ins2</i>	5'CCCTGCTGGCCCTGCTCTT3'	5'AGGTCTGAAGGTCACCTGCT3'
<i>Pre-Ins1</i>	5'TGGCTTCTTCTACACACCCAAG3'	5'ACAATGCCACGCTTCTGCC3'
<i>Pre-Ins2</i>	5'GGGGAGCGTGGCTTCTTCTA3'	5'GGGGACAGAATTCAGTGGCA3'
<i>gfp</i>	5'AGAACGGCATCAAGGTGAAC	5'TGCTCAGGTAGTGGTTGTC3'

## Supplemental Information

### Estimation of mRNA to protein delays

We estimate that the delay from *gfp* mRNA to fluorescent protein is about 1.3 hours (17.5 min for nuclear export + 0.5 min for initiation + 1 min for elongation + 60 min for maturation). In estimating the delays between transcription, translation and maturation we used the following information:

**Transcription rate:** 6-70 nucleotides/second (Darzacq et al., 2007; Singh and Padgett, 2009). Thus for GFP, which is 1788 nucleotides, it would take between 25 seconds and 5 minutes.

**Splicing time delay:** 30 seconds-10 minutes (Singh and Padgett, 2009)

**Nuclear export time delay:** 5-30 minutes (Oeffinger and Zenklusen, 2012)

**mRNA half-life:** For wildtype GFP, the mRNA half-life has been reported to be 7 hours (Sacchetti et al., 2001).

**Translation time delays:** Translational initiation takes 15-60 seconds and elongation completes 3-6 amino acids / second. Thus for GFP, which is 238 amino acids, we would expect ~40-80 seconds (Gerashchenko et al., 2019; Ingolia et al., 2011; Yan et al., 2016).

**Maturation delay:** The maturation of wildtype GFP takes 36-84 minutes (Balleza et al., 2018).

**Protein half-life:** The protein half-life of wildtype GFP is reported to be 54 hours (Sacchetti et al., 2001).

## References

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