fite treatment and sequencing[9] of the promoter area with the highest CG content did not reveal differences among cell lines expressing or not expressing *CASP8* (Fig. 1*g*). This indicates that methylation of this promoter is not associated with *CASP8* silencing. However, experimental evidence indicates that 5Aza-Cytidine upregulates *CASP8*. To address this problem, we transfected the *CASP8*$^-$ cell line SH-SY5Y with the pBL-CAT3-reporter vector bearing the novel promoter sequence. Demethylation of the transfected cells with 5Aza upregulated the endogenous *CASP8* and induced CAT expression (Fig. 1*h*). Because the transfected plasmids lack 5-methylcytosine, these results suggest that the generalized demethylation[10] of the cellular genome may upregulate *CASP8* through transacting factors.

In conclusion, we have cloned a DNA fragment at the 5′ terminus of *CASP8* that has promoter activity only in NB cell lines expressing this gene and whose activity can be indirectly modulated by demethylating agents. To our knowledge, this element represents the first functionally identified *CASP8* promoter. Its detailed functional analysis will provide new insights on the mechanisms that regulate this crucial apoptotic gene.

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Competing interests statement

The authors declare that they have no competing financial interests.

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Teitz et al. reply—Our previous manuscript[1] agrees with Banelli *et al.* on essentially all major points. In particular, we agree that there is minimal or no expression of caspase-8 in a substantial portion (25–35%) of NB cell lines and patient samples[1]. Furthermore, as we also described, Banelli *et al.* and others have observed a strong correlation between methylation of the *CASP8* 5′ UTR and *CASP8* expression in more than 30 NB cell lines and 150 NB patient samples[2,3,11–14]. Thus, methylation of this region could potentially be used as a diagnostic tool in conjunction with protein analysis, given that a few examples of discordant protein and RNA expression have been described (by Banelli *et al.* above and others[14]).

We also observed higher levels of *CASP8* methylation in *MYCN*-amplified and over-expressing cell lines and patient samples[1,3]. In particular, we demonstrated partial methylation in stage 1, 2 and 3 NBs, whereas complete methylation occurred almost exclusively in stage 4 NBs, especially those with amplified *MYCN* (67%)[1]. We do not yet know the biological significance of this observation.

We recognize that the region of *CASP8* we analyzed was not a classical CpG island and that it was not the promoter, as others have identified *CASP8* exons 5′ upstream to this sequence[14,16]. Thus, we described this sequence as a CpG-rich region and 5′-flanking sequence. Nevertheless, the correlation between the loss of expression and methylation of this region is notable and suggests a role for these sequences in *CASP8* silencing. Whereas the ability of 4HPR and IFN-γ to trans-activate *CASP8 in vivo* is certainly of interest[16], until more is known about the regulation of caspase-8 expression it is not necessarily inconsistent with the possibility that gene silencing involves a mechanism associated with this methylated region.

In contrast to Banelli *et al.*, we interpret the studies cited in their letter[2,3] as consistent with our results. Moreover, there may be a mechanism for the functional inactivation of caspase-8 in NBs expressing the protein, emphasizing the potential importance of caspase-8 in NB tumorigenesis[2].

Notably, caspase-8 expression and methylation of the *CASP8* 5′ UTR region have also been tightly linked to other neural crest tumors, including rhabdomyosarcoma, medulloblastoma, retinoblastoma and neuroendocrine lung carcinomas[14]. Silencing of *CASP8* in a variety of neural crest tumors warrants further study of the associated mechanisms.

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